International Journal of Agriculture and Biosciences 2025 14(1): 100-108.
https://doi.org/10.47278/journal.ijab/2024.195

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Isolation of *Bacillus thuringiensis* Strains to Create a Biological Pesticide for Agricultural Development and Environmental Sustainability

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ABSTRACT

This study aimed to isolate and identify highly virulent *Bacillus thuringiensis* (Bt) strains to develop biopesticides. Extensive field studies were conducted in nature reserves and woodlands to collect samples from soils and dead insects showing symptoms of bacterial infection. A total of 530 samples were collected, from which 30 Bt isolates were obtained and subjected to detailed physiological and biochemical analysis. The isolates were identified serologically and divided into three subspecies: *Bt kurstaki*, *Bt sotto*, and *Bt toguchini*. The biological efficiency of these strains was evaluated against eight species of lepidoptera pests in controlled laboratory conditions. Strains k-Ym07/CB and 2127-3k demonstrated the highest biological activity, reaching 100% mortality of the target insect species within five days. The results show that *Bt kurstaki* strains k-Ym07/CB and 2127-3k have significant potential as biological pesticides for controlling lepidoptera pests in Kazakhstan. The study concludes that developing these biological pesticides can offer an environmentally sustainable alternative to chemical pesticides, increasing agricultural productivity while maintaining an ecological balance.

Keywords: Biological efficiency, Bacillus thuringiensis strains, Sustainable ecology

Article History
Article # 24-859
Received: 02-Oct-24
Revised: 28-Oct-24
Accepted: 03-Nov-24
Online First: 27-Nov-24

INTRODUCTION

In recent decades, issues related to the production of environmentally safe agricultural products have become more relevant worldwide. One of the major challenges in achieving this goal lies in plant protection against arthropod pests, traditionally controlled primarily through chemical methods. However, the widespread use of synthetic pesticides leads to the accumulation of toxic substances in food products of plant and animal origin, a disturbance of the ecological balance in ecosystems, the appearance of resistant forms of pests and harmful effects on non-target fauna (entomophages, pollinators, birds, fish, etc.) (Monastyrskii, 2008; Kandybin et al., 2009). A significant reduction in pesticide pressure can ensure the use of environmentally safe methods to control the number of arthropod pests, including microbiological preparations. In Kazakhstan, research on biological solutions for crop pest management remained limited until the early 21st century (Alpysbayeva et al., 2018; Mukhamadiyev et al., 2023).

Using biological preparations in agriculture has several advantages, as they are safer for the environment and human health since they do not contain toxic substances. They are more specific in their actions (aimed only at pest species), without damaging beneficial insects and other living organisms. Biological pesticides cause less development of pest resistance since they act on them using various mechanisms (Monastyrskii, 2008; Thomsen et al., 1999; Belousova & Dolzhenko 2017; Crickmore et al., 2020).

However, the use of biological preparations in agriculture is still limited by some factors. One of them is the high cost of producing biological preparations, as they require special equipment and controlled conditions. There is also a problem of integrating biological preparations with other pest control methods, such as physical methods and resistant plant varieties (Monastyrskii, 2008; Kandybin et al., 2009; Grabova, 2015; Nugmanova, 2017; Shternshis, 2016; Alimbekova et al., 2021; Chadinova et al., 2020).

Phytoprotective products based on microorganisms play a special role in environmentally friendly farming systems. About 1% of the global market for plant protection products is made up of biological pesticides. A special place among the microorganisms that biological preparations are based on belongs to the entomopathogenic

Cite this Article as: Adilkhankyzy A, Tleubergenov KM, Shissenbayeva NZ, Uspanov AM and Duisembekov BA, 2025. Isolation of *Bacillus thuringiensis* strains to create a biological pesticide for agricultural development and environmental sustainability. International Journal of Agriculture and Biosciences 14(1): 100-108. https://doi.org/10.47278/journal.ijab/2024.195

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bacteria *Bacillus thuringiensis* (Bt) (market share of biopesticides: 90-95%) (Mnif & Ghribi, 2015; Haidar et al., 2016; Patel et al., 2013; Raymond & Federici, 2017; Tleubergenov et al., 2024).

Bt representatives are widespread due to their ability to effectively adapt to extreme conditions. These bacteria are isolated from soil samples, diseased and dead insects. Over 70 varieties effective against phytophages from the Lepidoptera, Coleoptera, Diptera, and Hymenoptera orders have been identified. After plant treatment, Bt bacteria remain viable for a long time (Ermolova, 2016; Malovichko et al., 2018). The high biological efficiency of Bt is associated with antifeedant, teratogenic, and anti-reproductive properties. Pathovar A is active mainly against Lepidoptera, B against larvae of blood-sucking mosquitoes and midges and herbivorous mosquitoes (Diptera) and C against Coleoptera (Rovesti & Sgarzi, 2000).

Due to the presence of crystals of endotoxin, exotoxin, phospholipase C and spores, *Bt* exhibits entomotoxic, entomopathogenic, and metatoxic effects. Bacteria, penetrating the body of insects, cause diseases accompanied by septicemia. The parasite passes in large quantities into the hemolymph and enters the intestinal epithelium, where it multiplies intensively and causes the death of insects (Safronova et al., 2015). The antifungal activity of Bt is associated with the production and release of protease and chitinase, which lyse the cell walls of phytopathogenic fungi (Belousova & Dolzhenko, 2017). The contents of the hyphae of the fungus become a source of nutrition and energy for Bt. Lipopeptide cyclic antibiotics, which have received attention in recent years, are also responsible for the antagonistic effect. Bt can produce antibiotics of the polypeptide and aminoglycoside series, capable of suppressing the growth and development of pests (Saber et al., 2015).

The combination of different mechanisms of action in bacteria of the *Bacillus* genus (their strains can produce from 50 to 200 biologically active substances) creates the basis for the effective reduction of the number of pests. However, the range of biological pesticides is much smaller than chemical ones.

The development and production of biological products require active, high-performing source strains, which are identified from natural sources based on criteria such as manufacturability, bioactivity, and broad-spectrum efficacy. These biopesticides hold significant potential to reduce reliance on chemical pesticides, contributing to sustainable and environmentally friendly agricultural systems. Such advances can improve product quality, conserve biodiversity, and support sustainable rural development (Becher, 2001).

For the development and production of biological products, active source strains are needed, searched for in natural sources according to the criteria of manufacturability, activity, and spectrum of action. Biological preparations have great potential to reduce the use of chemical pesticides and create sustainable and environmentally friendly agricultural production systems. This will help to improve product quality, preserve biodiversity, and ensure sustainable rural development (Becher, 2001).

In Kazakhstan, studies have been conducted on the isolation and identification of effective microorganisms capable of controlling the number of insect pests on crops. These microorganisms were later used to create microbiological biological preparations (Alimbekova et al., 2021). As a result, biological preparations containing bacteria, fungi, and viruses that specifically target pests and plant pathogens were developed and tested. These biological preparations are environmentally friendly and do not harm the environment, animals, and humans. The purpose of this study was to isolate Bt bacteria, identify them, and select strains promising as producers of biological preparations with entomocidal action against pests.

MATERIALS & METHODS

In the course of this study aimed at collecting natural substrates and searching for dead insects with signs of bacteriosis, we conducted route surveys of ribbon forests of the State Forest Natural Reserves Ertis Ormany in the Pavlodar region and Semey Ormany in the Abai region (Kazakhstan). Sections of the Borovoye forest area in the Akmola region and forests of eastern Kazakhstan were also surveyed (Fig. 1).

In the Almaty region, the natural reserves of the Ile Alatau State National Park and the forest belts of the Kegensky, Enbekshikazakh, and Karasai districts were surveyed (Fig. 2).

These territories are distinguished by their flora and fauna diversity (Mussynov et al., 2014; Serekpayev et al., 2016). These sites were not chosen by chance, as they represent unique ecosystems that have not been affected by anthropogenic factors, such as pesticide load, deforestation, pollution, and urbanization (Fig. 3).

During the study, we also collected soil and vegetation samples, which allowed us to conduct further analyses for the presence of pathogenic microorganisms. We collected 530 samples, of which 498 samples were examined: 277 natural substrates (soil, leaf litter, tree bark) and 221 dead insects with signs of bacteriosis. 48 Bt isolates were obtained from the collected dead insects. The obtained isolates corresponded to Bt in physiological and biochemical properties. According to the results of serological identification, the bacteria we isolated were assigned to three serotypes: 3a3b3c, subspecies Bt kurstaki; H4ab, subspecies Bt sotto; and 31 serotypes of the species Bt toguchini (Table 1).

Table 1: Nucleotide sequence of primers used for *Bt* strain genotyping

Primer number	Nucleotide sequence	Amplicon mass, Nucleotide bases
BOX (boxA, boxB, boxC)	5′-CTACGGCAAGGCGACGCTGACG-3′	154
gyrB-F1	5′-ATGGAACAAAAGCAAATGCA-3′	1,883
gyrB-R1	5′-TTAAATATCAAGGTTTTTCA-3′	

The identification of the obtained isolates using the classical method was carried out according to the bacterial determinant together with the staff of the Institute of

Fig. 1: Surveyed natural reserves in Kazakhstan.

Fig. 2: Surveyed natural reserves and forest belts in the Almaty region.

Fig. 3: State natural reserves of Kazakhstan.

Systematics and Ecology of Animals at the Siberian Branch of the Russian Academy of Sciences (SO RAN) in Novosibirsk, Russia. Identification by polymerase chain reaction (PCR) analysis was carried out at the All-Russian Institute of Plant Protection (VIZR) in Pushkino, Russia. The cultural and morphological properties of bacteria were studied on dense nutrient media of various compositions: medium A made of peptone: 1%, fish hydrolysate: 0.4%, NaCl: 0.5%, agar-agar: 1.5-2.0%, H_2O: 100 mL; nutrient agar made of fish hydrolysate: 0.4%, NaCl: 0.5%, agar-agar: 1.5-2.0%; meat infusion agar made of meat infusion broth: 100mL, agar-agar: 1.5-2.0%, non-nutrient agar: 1.5-2.0%, H_2O: 100mL

To study the properties of colonies, bacteria were cultured in Petri dishes on medium A. Colonies were characterized by the following characteristics: size, shape, transparency, edge contour, relief, surface, color, structure, and consistency (Becher, 2001). To assess the specificity of the action of spore-crystal mixtures on insects, the strains were grown on medium A for 6 days at 30°C, until the spores and crystals completely appeared.

The biochemical activity of bacteria was studied by the nature and quantity of those enzymes that the microbial cell produced and released into the external environment. To diagnose microbes, determining saccharolytic and proteolytic enzymes that activate the breakdown of carbohydrates and proteins is of the greatest importance (de Barjac & Bonnefoi, 1967)

To detect saccharolytic enzymes, the studied bacterial culture was seeded into a nutrient Hiss's medium (1% peptone, 0.5% NaCl, 0.5% carbohydrate, 1mL of Andrade indicator). The medium was used to test the behavior with sucrose, salicin, and mannose. Inoculation and incubation were performed at 28°C. A reaction was considered positive based on the redness of the medium.

Acetylmethylcarbinol (AMC) was determined using the Voges-Proskauer test (de Barjac & Bonnefoi, 1967). The lecithovitellin reaction (LVR) was performed according to Becher's method (Bekher, 1961). Catalase was determined using I.I. Ashmarin's method. Urease was determined using Christensen's medium. Starch hydrolysis was detected on medium A with the addition of 0.2% soluble starch. The medium was inoculated by injection. After incubation, the agar was filled with Lugol's solution. A reaction was deemed positive based on the appearance of a colorless area around the growth zone. Proteolytic properties were determined by hydrolysis zones during spot seeding on casein agar (Labinskaya, 1978). A reaction was deemed positive based on a colorless area around the colony growth zone.

***Serology*:** The serological identification consists of the following stages: obtaining the antigen of the culture and typical cultures, preparing antiserum dilutions, and conducting an antigen agglutination reaction with an antiserum of the appropriate dilution.

To study the antigenic structure of crystal-forming bacteria isolated from nature, specific intraspecific serums were used, which were located in the museum of the Laboratory of Insect Pathology of the Institute of Systematics and Ecology of Animals, SO RAN.

The flagellar H-antigen of typical cultures and isolated native strains was obtained using the methods developed by de Barjac and Bonnefoy (1967), Talalaeva and Pokrovskaya (1978) and Pokrovskaya (1982).

RESULTS

During the route surveys, dead caterpillars of the apple ermine (Hyponomeuta malinella), leaf-rolling moth (Tortricidae), owlet moth (Noctuidae), turnip butterfly (Pieris rapae), etc. were found, presumably with signs of bacteriosis (Fig. 4). In Fig. 4, the insect dried out and shriveled, while the cuticle remained intact; the internal tissues were viscous and often had an unpleasant odor. Infected insects were primarily collected from areas where mass outbreaks had been observed. Naturally deceased insects were located in environments such as spider webs, curled leaves, and similar sites. Most of the dead insects were collected in the Ile-Alatau State National Natural Park and the Yertis Ormany ribbon forests. Some forestry areas in these regions were characterized by a high level of insect pests. Mass epizootic outbreaks of the pine tree moths on pine trees and apple ermine on Asian wild apple trees were observed there (Fig. 5). The smallest number of dead insects were found in the forests of the Akmola region (Fig. 6). Dead caterpillars with signs of bacteriosis were identified as caterpillars of leaf-rolling moth (Tortricidae), gypsy moth (Lymantria dispar), mottled umber moth (Geometridae), apple ermine (H. malinella), pine tree moth (Dendrolimus pini), etc.

During sporulation, crystals of oval, diamond, and bipyramidal shapes were observed (Fig. 7). As a result of microscopy, we found that vegetative cells were represented by large rods located separately or in chains in the smear preparation the spores were oval. Complete formation of spores and crystals occurs on day 5 when cultivated in a thermostat at 28-30°C.

Determination of the Range of Sensitive Insect Pests

An important element in the development of biological pesticides is to determine the range of insect pests sensitive to strains of entomopathogenic bacteria.

We evaluated the biological efficiency of 30 bacterial strains on eight species of pests from the Lepidoptera order: *H. malinella*, *Archips crataegana*, *Archips rosana*, *Erannis defoliaria*, *Operophtera brumata*, *Malacosoma neustria*, *Hyphantria cunea*, and *Pieris brassicae*. Observations showed significant variability of strains based on virulence. Eight bacterial cultures (k-Ym07/KOKh, k-Ym07/CB, OZSH-07, k-Ym07/K, ZPT-07, OZSh-07/1, k-Ym07/ZR1, k-Ym07/AK) showed high biological activity against apple ermine caterpillars. On the 5th day after inoculation, the caterpillar mortality rate for these strains reached 90-100%. According to the final mortality rate and the rate of death of the host, k-Ym07/KOKh, k-Pr07, k-Ym07/CB, k-Ym07/K, k-Ym07/AK were the best in this sample of cultures. For these strains, from 90 to 97.5% of infected specimens died on the 4th day after infection. The remaining cultures on the 4th day showed less biological activity (up to 90%).

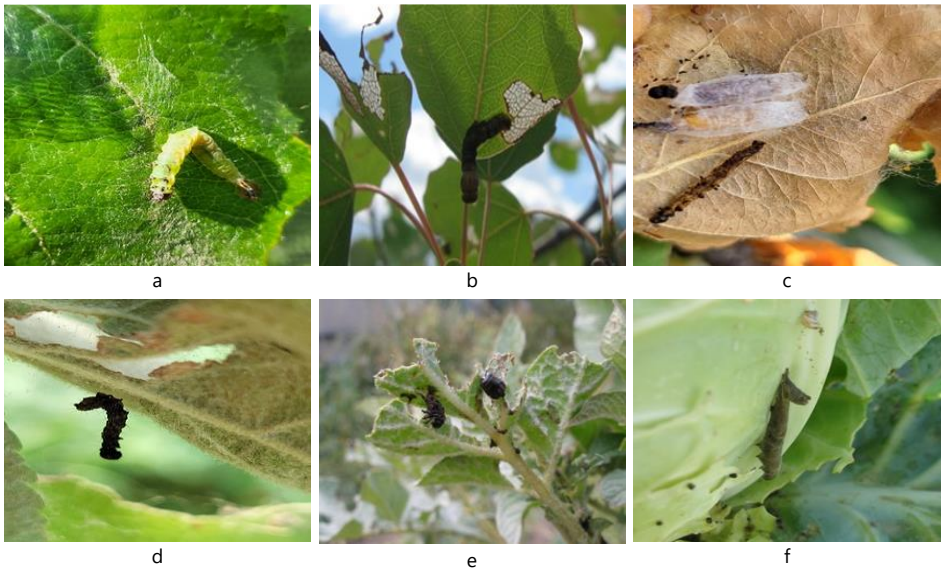

Fig. 4: Dead insect larvae with signs of bacteriosis; a: *Archips crataegana*; b: *Pheosia tremula*; c: *H. malinella*; d: *Helicoverpa armigera*; e: *Leptinotarsa decemlineata*; f: *P. rapae.*

Fig. 5: Pest distribution in forest areas: a. *Dendrolimus pini*; b. *H. malinella.*

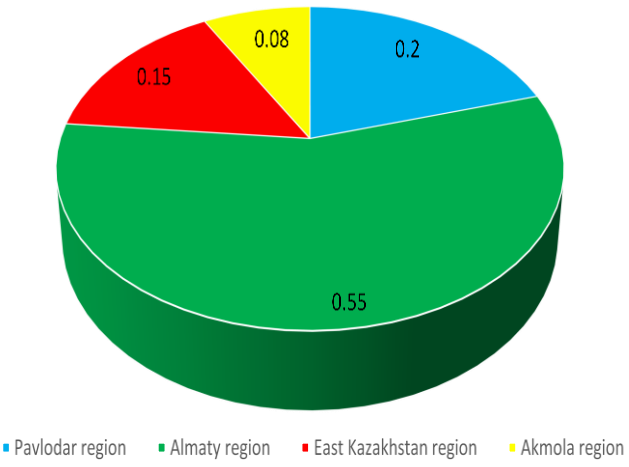

Fig. 6: Isolates obtained from dead insects.

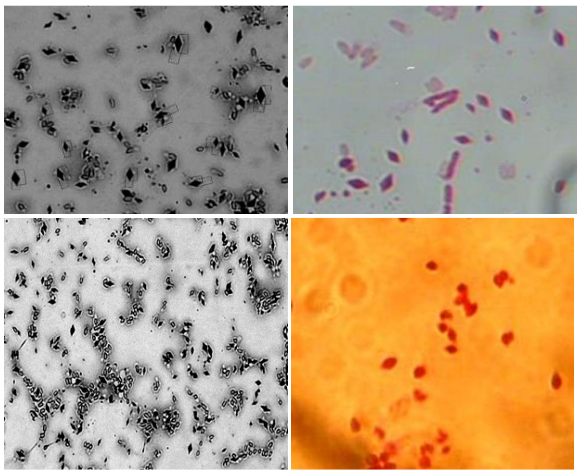

Fig. 7: Micromorphology of the crystal-forming bacteria of the *Bt* group.

Similar experiments have also been conducted on the caterpillars of the American white moth. Like in the experiments conducted earlier concerning apple ermine caterpillars, not all strains achieved high results in these experiments. Strains 4Cr-06, k-Ym07/ZR, Kzh-07, Kzh-07/1, ChM-07, ChM-07/1, k-Ym07/ZR, ChM-07/4, SSH-1/07 and k-Ym07/ZR-3 proved to be low-virulent for the American white moth caterpillars. On the 5th day, the strains k-Pr07 and k-Ym07/KB and the reference strain used for the Kazakh preparation Aq köbelek 2127-3k resulted in 100% death of the caterpillars infected with a titer of $1x10^7$ spores/mL. According to the final mortality rate and the rate of death of the host, k-Ym07/KB rendered the best results in this sample of cultures, as with its use the mortality rate reached 100% as early as the 4th day. The virulence of the remaining strains has reached more than 70%.

Strains of the *Bt* bacterium were less virulent against the caterpillars of the American white moth. Thus, when the pest was infected, only five cultures (k-Ym07/KoKh, Ag-07p, k-Pr07, k-Ym07/CB, k-Ym07/ZR) out of 30 showed high biological activity (90-100%). The number of medium-virulent strains was 40%, and the number of low-virulent strains was 43.3%.

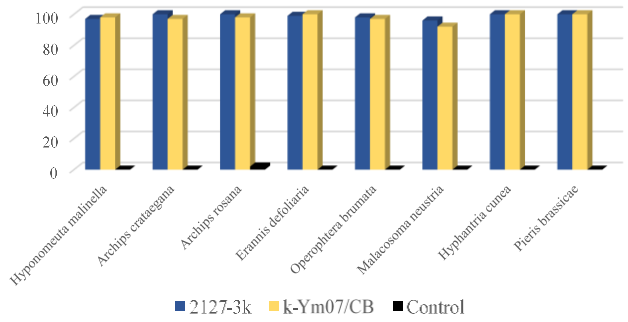

Fig. 8: Evaluation of the efficiency of strains of the entomopathogenic bacterium *Bt* against caterpillars from the Lepidoptera order.

The k-Ym07/KOKh, k-Pr07, and k-Ym07/CB strains had the highest biological activity against the caterpillars of brown oak tortrix and mottled umber moth. The mortality rate of the caterpillars was 85, 92 and 95%, respectively. Of all the strains, the maximum biological activity against pests from the Lepidoptera order was shown by k-Ym07/CB and 2127-3k (standard) (Fig. 8).

Molecular Genetic Studies of *BT*

To conduct molecular genetic studies to develop a genotyping system for *Bt* strains, DNA analysis methods based on (a) sequencing of informative genome loci and (b) determination of amplification profiles with a random primer of the BOX system were used. Cultures of toxin-forming bacteria strains k-Ym07/KB and 2127-3k and non-toxin-forming bacteria OspSib-1 and Z1 isolated from various sources were used as prototypes (Table 2). DNA extraction was performed, followed by testing with degenerate primers targeting a section of the gyrase B gene (*gyrB*) exceeding 2,000 bp in length. Primers targeting a 600 bp region of the 16S ribosomal RNA gene served as an internal control (Table 3). A qualitative positive signal for internal control confirmed successful DNA extraction and sample suitability for molecular analysis. However, multiple initial attempts to establish PCR conditions for the *gyrB* locus were unsuccessful, suggesting the need for further optimization. This includes selecting primers targeting a shorter fragment of the *gyrB* gene to facilitate reliable genotyping of the strains. Amplicons obtained with primers for the 16S RNA gene for all four strains were sequenced. The sequences of strains AK1 and L1 showed belonging to a single molecular haplotype characterizing a group of closely related species of Bt. Similarly, the identical sequences of the Z1 and OspSib-1 strains belonged to another haplotype peculiar to the group of species including *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformes,* etc. These two haplotypes were characterized by a significant level of divergence since their similarity did not exceed 96%.

Table 2: Physiological and biochemical characteristics of *Bt* bacterial strains

No.	Strains*	Serotype	Subspecies	AMC	Urease	LVR	Hydrolysis	Proteo-lysis	Catalase	Acid on		
										mannose	salicine	sucrose
1	k-Ym07/BB	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
2	k-Ym07/KB	3a3b3c	*kurstaki*	+	+	+	+	+	+	+	+	–
3	ChM-07/4	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	–	–
4	k-Ym07/ZR1	3a3b3c	*kurstaki*	+	–	+	+	+	+	–	–	–
5	ZPT-07	H4ab	*sotto*	+	–	+	+	+	+	–	–	–
6	OZSh-07	3a3b3c	*kurstaki*	+	–	+	+	+	+	–	–	–
7	OZSh-1/07	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
8	Ssh-107	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
9	Ssh-07	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	–	–
10	Kzh-1/07	H4ab	*sotto*	+	–	+	+	+	+	–	–	–
11	Kzh-07	H4ab	*sotto*	+	–	+	+	+	+	–	–	–
12	k-Pr07/1	3a3b3c	*kurstaki*	+	–	+	+	+	+	–	–	–
13	k-Pr07/2	3a3b3c	*kurstaki*	+	–	+	+	+	+	–	–	–
14	k-Pr07	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	–	+
15	k-Ym07/K	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	–	+
16	k-Ym07/KOH	3a3b3c	*kurstaki*	+	+	+	+	+	+	+	+	+
17	k-Ym07/ZR	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	–	+
18	ChM-07/1	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
19	ChM-07/2	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
20	k-Ym07/ZR2	3a3b3c	*kurstaki*	+	–	+	+	+	+	+	+	+
21	k-Ym07/ZR-3	3a3b3c	*kurstaki*	+	–	+	+	+	+	+	+	+
22	Hs-07/1	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
23	Hs-07/2	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
24	Hs-07/3	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
25	Hs-07/4	3a3b3c	*kurstaki*	+	–	+	+	+	+	+	+	+
26	ChM-07/3	3a3b3c	*kurstaki*	+	–	+	+	+	+	+	+	+
27	ChM-07	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
28	Hs-07/r	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
29	k-Ym07/AK	3a3b3c	*kurstaki*	+	+	+	+	+	+	–	+	–
30	4Gr-06	31	*toguchini*	+	–	+	+	+	+	+	+	–

Symbols: + the presence of an enzyme; – the absence of an enzyme

The amplification profiles according to the BOX system contained several distinctly separated bands and an incomplete separation zone for products with dimensions of about 100 nm (Fig. 10, track 4). Despite the difference in the intensity of band staining in different samples and at different dilutions of genomic DNA, the same profile can be observed for DNA samples of strains belonging to the same haplotype. Noticeable differences were observed in the profiles of two haplotypes (Fig. 9). The results differ significantly from the original data. According to the approved DNA extraction technique, genomic DNA samples of 16 strains were prepared for further studies of their genotypes.

Thus, based on the results of the first research stage, DNA samples were selected, and the need for further selection of markers suitable for molecular diagnostics with a higher level of precision (determination of species and serotype) was shown.

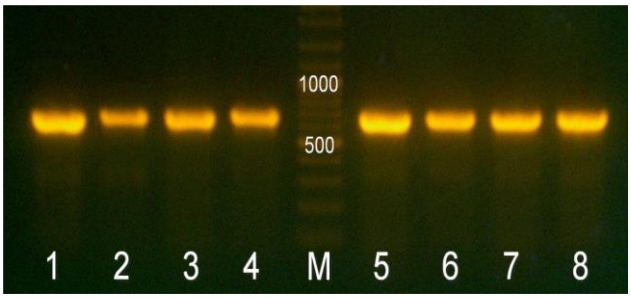

Fig. 9: Electrophoretic separation of amplifications of a fragment of the 16S rRNA gene with primers

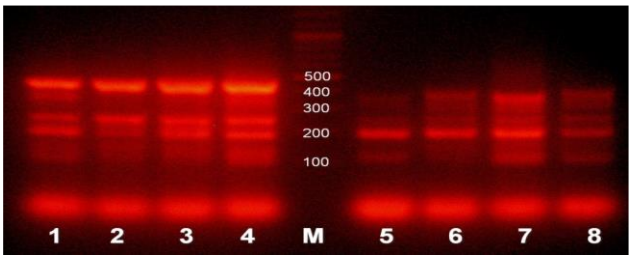

Fig. 10: Electrophoretic separation of amplifications of genomic DNA samples of strains.

Table 3: Results of identification by analysis of the nucleotide sequence of a fragment of the 16S rRNA gene using primers, %

	2127-3k	k-Ym07/KB	OSPS	Z1
2127-3k	identical	100.0	95.3	95.3
k-Ym07/CB	100.0	identical	95.3	95.3
OSPS	95.3	95.3	identical	100.0
Z1	95.3	95.3	100.0	identical

Electrophoretic separation of amplifications of a fragment of the 16S rRNA gene with primers (B,D)F|(B,D)R for strains AK1 (1,2), L1 (3,4), Z1 (5,6), and OspSib-1 (7,8) in 1% agarose gel, staining with ethidium bromide. The initial DNA samples were diluted 10 times (odd numbers) and 100 times (even numbers) and added to the PCR mixture in equal volume. M is a marker of the molecular weight of GeneRuler™ Thermo Scientific, the sizes of the reference bands are indicated in n.b. (Nucleotide bases).

Electrophoretic separation of amplifications of genomic DNA samples of strains 2127-3k (1,2), k-Ym07/CB (3,4), Z1 (5,6), and OspSib-1 (7,8) with BOX primer (da Silva, Valicente 2013) in 1% agarose gel, staining with ethidium bromide. The initial DNA samples were diluted 10 times (odd numbers) and 100 times (even numbers) and added to the PCR mixture in equal volume. M is a marker of the molecular weight of GeneRuler™ Thermo Scientific, the sizes of the reference bands are indicated in n.b.

DISCUSSION

The study of the potential use and mechanisms of action of Bt strains and the search and description of the properties of newly obtained isolates as possible agents in the biological control of pests and plant diseases are extremely relevant (Zafar et al., 2020).

Our results indicate the prospects of the k-Ym07/CB strain as a producer of a biological preparation against Lepidoptera pests. In our study, we isolated Bt strains from various natural substrates, soil samples, and diseased samples that have been unaffected by anthropogenic activities, creating a naturally derived Bt source; this method agrees with the work of (Sridhara et al., 2021), who emphasized the importance of the continuous exploration of new Bt strains from different ecological regions could be advantageous for Bt based bioformulations and the generation of transgenic plants. Our study also aligned with the work of KhM et al. (2024), who biotested Bt strains against the caterpillars of *Hyponomeuta malinella* in the Almaty region. Our results also consistent with the work of Sauka et al. (2022), Nascimento et al. (2024) and Choe et al. (2022), who concluded that specific Bt strains were able to display over 90% efficacy against *Hyponomeuta malinella, Tortricidae, Noctuidae* and *Pieris rapae*. Thus, our study confirmed that entomopathogenic crystal-forming *Bt* bacteria are found in soil, leaves, diseased and dead insects, and insect habitats.

Our results also consistent with (Sridhara et al., 2021), who emphasize the importance of pH and temperature of cultivation of Bt strains. Although the pH values in both studies were not stated, we can make a hypothesis that Bt strains thrive in neutral pH values; this does not agree with the work of (Birolli et al., 2021), who recorded a pH value of 8.5 as the optimal condition This creates a gap in the relation of different ecological niches, the pH of the sample and the performance of the strain.

For the biochemical analysis, our procedure agrees with the works of (Sujayanand et al., 2021), who subjected their strains to catalase, urease, starch-hydrolase, and Voges-Proskauer tests in diagnosing microbes; these tests appear to be relevant to current research trends due to their appearance in multiple studies (Sridhara et al., 2021) Our process also agrees with the work of (García-Suárez et al., 2021), in studying the antigenic structure of the crystal forming bacteria and flagellin (*hag*) gene sequencing.

Our study on the effect of Bt strains on the larvae of the American white moth agrees with the work of (Bayrak et al., 2023), who concluded in their study that in all the Bt strains tested, the mortality rate of the American white moth (*Hyphantria cunea*) increased with days and at large doses. This makes Bt biopesticides the best solution for

pest control as overexposure of chemical pesticides have shown to produce undesirable effects (Razzaq & Yang, 2023).

Identification and bioassay showed that the isolates were related to the sero-variants of *Bt kurstaki*. In terms of biological properties and practical significance, they were close to the typical strains of this subspecies. With the help of analytical selection, selection of nutrient media, and cultivation regimes, we enhanced the practically valuable properties of these isolates and successfully used them as producers of biological preparations to control the number of insect pests. Microbial preparations can compete with chemical pesticides in environmental safety and economic terms, considering the protective effect against pests and diseases.

Our results also prove the relevance and superiority of Bt strains from the kurstaki subspecies and agree with (Park et al., 2022) on the toxicity of the kurstaki subspecies to lepidopteran pests. Nonetheless, it is important to acknowledge that while Bt preparations have proven effective, they are not without limitations; as stated by (Ma, 2024), The development of resistance by crop pests to Bt toxins in organic farming is a significant limiting factor. This resistance could involve genetic mutations in the target pest to specific Bt strains or proteins. These resistances must be monitored to explore the genetic mechanism and develop strategies to counter these developments (Ren et al., 2019). Also, regulator and market challenges are limitations to the adoption of Bt in organic farming.

Research and development in microbiological plant protection in Kazakhstan is necessary. It is necessary to conduct systematic research on new effective biological products, improve their quality, and adapt to local conditions. It is also important to cooperate with international scientific organizations and other countries to share experience and transfer the latest technologies. Considering the massive introduction of legal norms regulating the use of pesticides and environmental protection, biological preparations are becoming an environmentally sound alternative and an economically viable solution to increase yields and reduce plant protection costs.

Conclusion

The obtained results indicated that among the isolated and studied strains of Bt, 86.6% were attributed to Bt *kurstaki* (3a3b3c). Most of them were highly effective against some pests from the Lepidoptera order (*H. malinella, A. crataegana, A. rosana* etc.). These findings provide key indicators for developing biological insecticides with multiple strains of the same serotype. Following screening against eight Lepidoptera species, we identified one strain, k-Ym07/CB, as a promising candidate for the development of a locally produced biological pesticide in Kazakhstan.

Acknowledgment: The study was carried out within the framework of the grant funding with the individual registration number (IRN) AP14871184 "Creation of a made-in-Kazakhstan biological insecticide based on the Bacillus thuringiensis bacterium for the control of Lepidoptera pests in Kazakhstan".

Author's Contribution: A.A. conceptualized and designed the study, led the experimental work, and wrote the initial manuscript draft. K.M.T. and N.Z.S. contributed to sample collection, laboratory experiments, and data analysis. A.M.U. provided guidance on methodology, assisted in strain identification, and participated in data interpretation. B.A.D. supervised the project, secured funding, and critically revised the manuscript for intellectual content. All authors have reviewed and approved the final version of the manuscript for submission.

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