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ARTICLE

Screening of bacterial strains for potential biopesticidal activity against the forest pest Jacobson's spanworm (Geometridae, Erannis jacobsoni Djak.)

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Abstract: This study focused on developing a biological control agent targeting Jacobson's spanworm (Erannis jacobsoni Djak.), a major forest pest in Mongolia. The aim of the research was to isolate and identify *Bacillus thuringiensis* strains capable of producing toxic crystal proteins, with the goal of contributing to biopesticide development. Samples were collected from dead insects showing symptoms of bacterial infection in order to isolate and screen bacterial strains with activity against Jacobson's spanworm. Among them, isolate MNL 78-4 demonstrated the highest effectiveness, causing 100% mortality of target insects within 8 days. This result suggests that MNL 78-4 is a promising candidate for further development as a biological control agent for managing Jacobson's spanworm in Mongolia. These findings indicate that eco-friendly microbial pesticides may provide a sustainable alternative to synthetic chemical insecticides, contributing to forest health, biodiversity conservation, and ecosystem balance.

Keyword: *Jacobson's spanworm, Bacillus thuringiensis, biopesticides, forest pest;*

INTRODUCTION

Global demand for agricultural products manufactured using environmentally sustainable methods is growing. A significant challenge in meeting this demand is protecting crops from insect pests, which are still controlled mainly with chemical pesticides. However, the application of chemical pesticides has adverse impact on food safety, disrupts the ecosystems, complicates the management of pest populations, and jeopardises beneficial organisms within the ecosystem [1].

Erannis jacobsoni Djak (E. jacobsoni) it is a major forest pest in the Mongolian Plateau, causing severe damage to forests. Since 1920, it has spread across Mongolia, and the infested areas have increased, making it the most destructive larch pest since 2020. Recent outbreaks are also spreading in northern and northeastern Mongolia, with a potential risk of further spreading [2,3,4].

This pest produces one generation per year and over winters the generation is in the egg stage.

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Larvae of *E. jacobsoni* emerge in early May and begin intensive feeding by early June. After four molts, they enter the soil to pupate by late July. Adults typically appear in September, and infestations may continue until mid-October. The females are wingless and incapable of dispersal; they lay their eggs in bark crevices and overwinter with them, allowing repeated infestations of the same trees over several consecutive years.

E.jacobsoni exhibits an annual cycle that can culminate in severe outbreaks, after which affected trees may experience leaf loss for 3–4 years, ultimately leading to their mortality. When this pest spreads, it depletes key biochemical reserves, including chlorophyll, water, and nitrogen. As the tree weakens, its colour fades, leaves shed more rapidly, and even the way it reflects light begins to change [5,6].

Currently, pest control in Mongolia relies primarily on manual pesticide application. These measures are largely experience-based and do not distinguish between varying levels of pest distribution. As a result, heavily infested areas often receive insufficient treatment, while lightly affected regions are exposed to excessive pesticide use, leading to environmental pollution [7,8]. Therefore, it is critical to adopt new approaches for monitoring the spread of forest pests and diseases in order to reduce their ecological impact and associated economic losses. The use of biological products in agriculture has the advantage of being safer for the environment, and for human health as well, because of their non-toxic nature. In addition, the use of biopesticides, derived from local bacterial strains, is environmental-friendly, and offers a promising alternative for sustainable pest management. This approach not only enhances crop protection but also encourages biodiversity, reduces dependence on synthetic chemicals, and thereby, supports healthy ecosystems [9]. They are more specific in their actions, targeting only pest species without harming beneficial insects or other organisms. Biological pesticides also cause less development of pest resistance, as they affect pests through multiple mechanisms [10]. A special place among the microorganisms used in biological preparations is held by entomopathogenic bacteria, such as *B.thuringiensis*, which dominate the biopesticide market [11]. The use of local *B.thuringiensis* strains as biopesticides reduces ecological risks by providing increased protection against specific pests [12]. The *B.thuringiensis* is a gram-positive, spore-forming bacterium known for producing crystal protein, and is widely used as a microbial insecticide, which is toxic to specific insect orders. The *B.thuringiensis* toxin disrupts epithelial cells in the insect midgut by acting on their exterior, penetrating the plasma membrane without entering the cytoplasm, ultimately causing cell lysis [13].

In previous studies, *B.thuringiensis* has been extensively investigated as a biopesticide against insects. For instance, researchers reported that Lepidoptera pests decreased significantly after using *B.thuringiensis* strains isolated from nature reserves and woodlands, which have significant potential as a biological pesticide for controlling *Lepidoptera* pests [14,15]. Local bacterial strains are naturally better adapted to their native climate and environment, enabling them to remain active longer and work more effectively. This makes it crucial to identify, test, and develop such strains to control *E.jacobsoni*, a rapidly spreading forest pest in Mongolia. Because the spread and impact of this pest depend on factors, such as forest conditions, air quality, soil, and temperature, identifying effective native strains and developing practical biopesticide solutions are crucial for sustainable forest protection. Despite Mongolia's unique biodiversity and environmental conditions, little research has focused on isolating and developing such strains for controlling insects, revealing a significant gap in pest management knowledge. This study aimed to isolate native *B.thuringiensis* strains active against insects, test their effectiveness in the

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laboratory, and explore their potential for sustainable use as biopesticides in Mongolia.

MATERIALS AND METHODS

Sample collection

This study involved collecting natural substrates and searching for dead insects exhibiting signs of bacteriosis across various locations in Mongolia. The surveyed sites included Ulaanbaatar city (Khan-Uul district, 23rd khoroo, Khuushiiin Am; and Bayanzurkh district, 11th khoroo, Tur Khurkhiin Am), Arkhangai *aimag* (Chuluut *soum*), and Arkhangai *aimag* (Ikh Tamir *soum*) shown in Table 1. and Figure 1.

Table 1. Sample collection sites.

	Codes of pure bacterial isolates	Collection site	Date of collection
1	MNL 36	Ulaanbaatar city, Khan-Uul district, 23rd khoroo, Khuushiiin Am	2024.07.08
2	MNL78-2, MNL78-3, MNL78-4, MNL HA	Ulaanbaatar city, Khan-Uul district, 23rd khoroo, Khuushiiin Am	2024.07.08
3	MNL 81-1 to MNL 81-4	Ulaanbaatar city, Bayanzurkh district, 11th khoroo, Tur Khurkhiin Am	2024.08.01
4	MNL Ap-1-1	Arkhangai aimag, Chuluut soum	2024.06.05
5	MNL Ap-21-1, MNL Ap-21-2	Arkhangai aimag, Ikh Tamir soum	2024.07.07

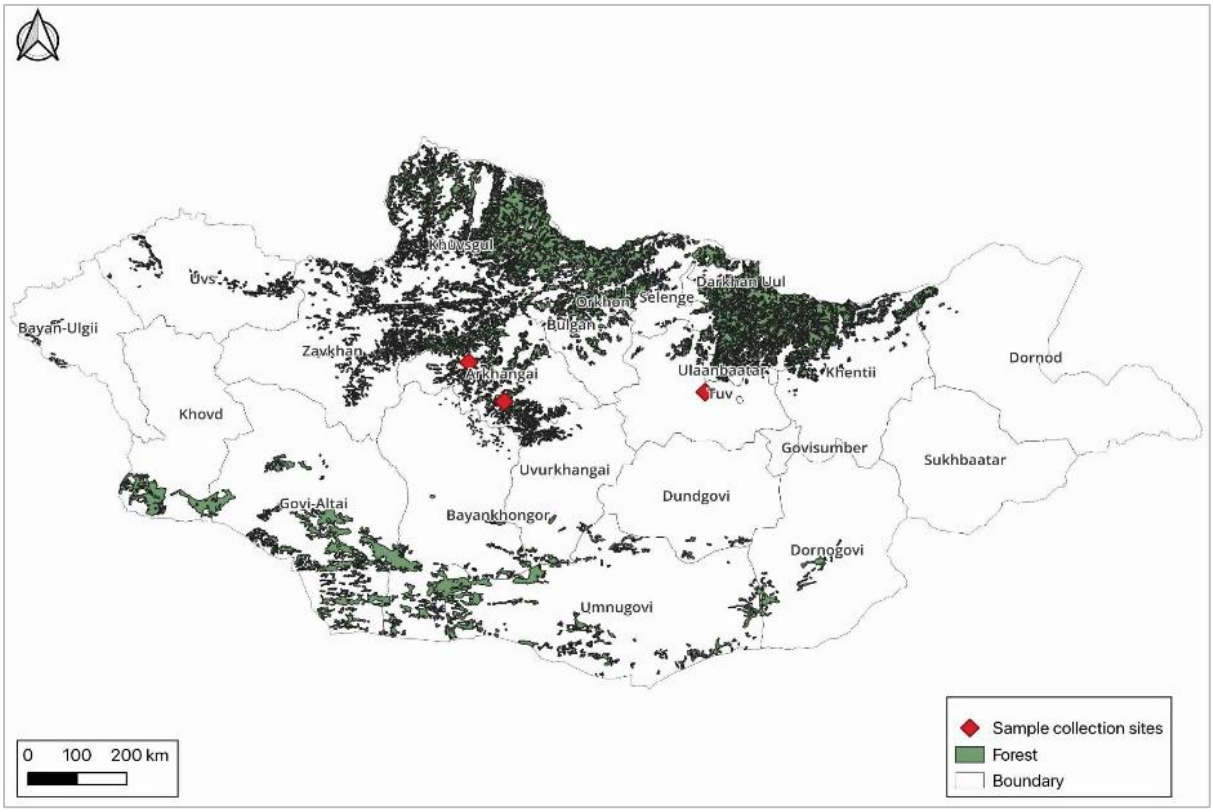

Figure 1. Sampling sites in Mongolia

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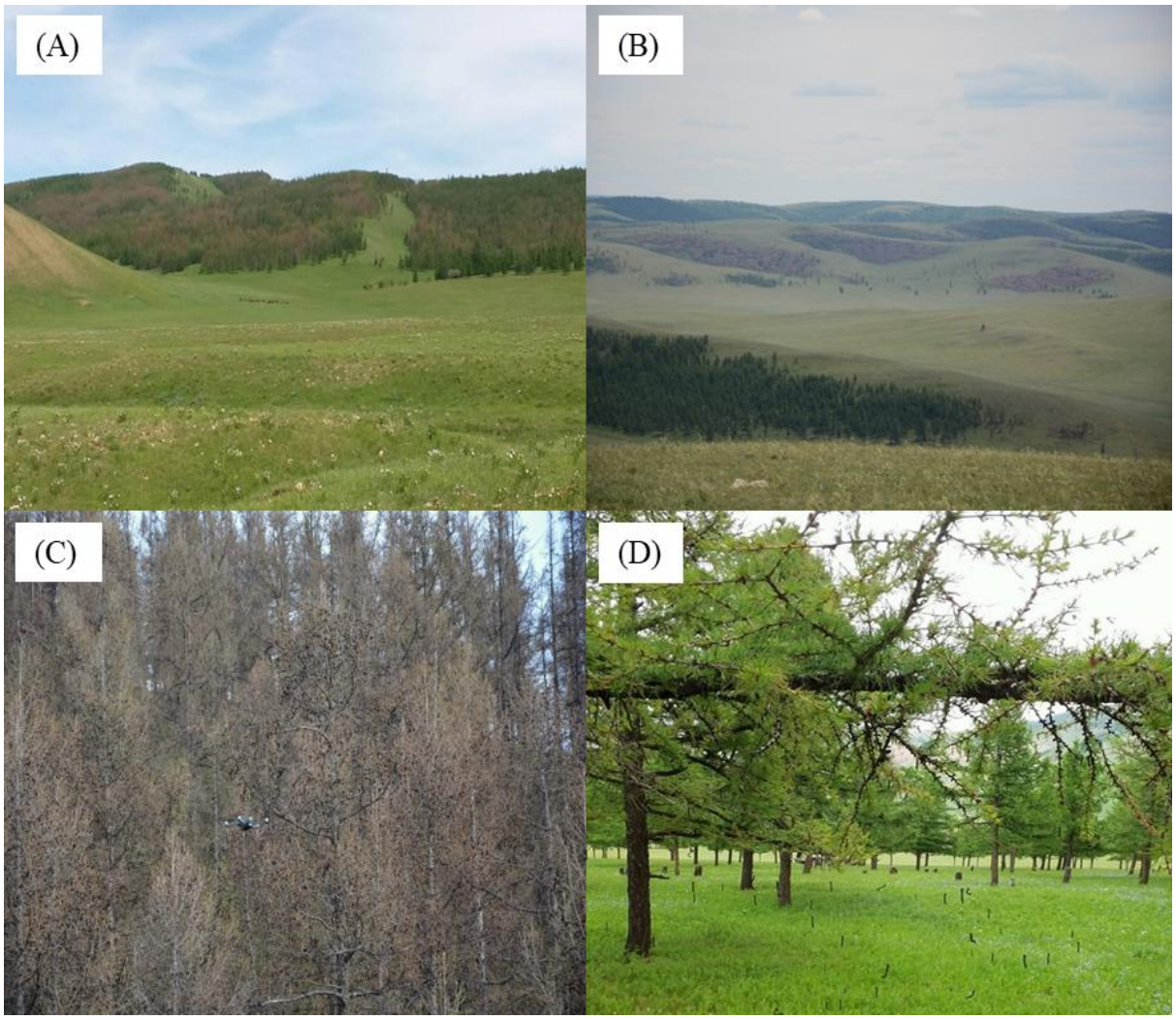

Figure 2. Representative forest landscapes in Mongolia affected by Erannis jacobsoni Djak. (A) Shivert, Battsengel soum, Arkhangai aimag, (B) Azarga Pass, Binder soum, Khentii aimag; (C) Azarga Pass, Binder soum, Khentii aimag; (D) Shivert, Battsengel soum, Arkahngai aimag.

Isolation of native *B. thuringiensis* strains

Larval cadavers of Jacobson's moth were collected from natural forests in Mongolia, in areas where no pest control measures had been applied, and transported to the laboratory under sterile conditions. Approximately 10 g of larval tissue was suspended in 90 ml of sterilised distilled water and incubated at controlled temperatures for 30, 60, and 90 minutes. From each suspension, 1 ml aliquots were withdrawn and subjected to serial dilution up to 10^{-7}. Aliquots of 100 µl from the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were spread onto Petri dishes containing nutrient agar medium and incubated at 37°C for 24–48h. Nutrient agar was prepared by dissolving 5g of peptone, 3g of meat extract, 5g of NaCl, and 15g of agar in 1,000 ml of distilled water. The pH was adjusted to 7.4 prior to sterilisation by autoclaving at 121°C for 15 minutes. Distinct bacterial colonies were subsequently selected and purified to obtain pure cultures.

Identification and morphological characterisation

Gram staining and the catalase test were conducted in order to initially identify the bacterial isolates. All bacterial isolates showed the typical features of the genus *Bacillus*, including Gram-positive, catalase-positive, rod-shaped cells [16]. A key step in the presumptive identification of *B.thuringiensis* was the search for parasporal crystals in sporulating cultures, since these protein inclusions distinguish *B.thuringiensis* from closely related species [17]. The bacterial isolates were incubated on

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NA at 37 °C for 48-72 h to promote sporulation, then stained with Coomassie Brilliant Blue and examined under a phase-contrast microscope following the protocols described previously [18].

Morphological features were documented both at the colony and cellular levels. On LB agar plates when they were incubated for 24–48 hours, we recorded colony traits, including size, pigmentation, surface texture, and margin type [16]. At the cellular scale, Gram staining and phase-contrast microscopy at 1000× magnification revealed cell shape, sporulation patterns, and the presence of crystal inclusions [19].

Collection and identification of larvae for testing in the laboratory

Larvae of *E.jacobsoni* were collected from their natural habitats, primarily from host plants and surrounding foliage, during the peak activity period. Sampling was conducted in the morning (08:00–12:00 hours) to coincide with the larvae's active feeding time. Individuals were gently removed from leaves and stems using fine forceps or a soft brush to minimise physical damage. Each specimen was placed in a labeled vial (25–50 mL) containing a small portion of fresh host plant material to maintain a natural microenvironment during transport. Each experiment was conducted in three independent replicates (n=3), using larvae as the experimental units. The vials were kept in a cool, shaded container to reduce stress and preserve larval viability until laboratory processing.

Collected larvae were first identified based on external morphological characteristics, including body length, coloration, head capsule shape, and the presence of distinctive markings or setae [20]. The identification followed standard taxonomic keys and reference materials, such as the Insect Larvae Identification Guide and Moths and Butterflies of Northern Asia. Each specimen was assigned a unique code, along with the collection date and site, to ensure traceability throughout the study.

Larvae exhibit a pale yellow to green coloration on the thoracic and abdominal segments, as shown in Figure 3. Second-instar larvae possess a brownish head capsule with pale brown spots and a distinct black dorsal stripe. The body is primarily yellow with several dark brown markings. Larval coloration gradually changes with each successive molt. The larval stage occurs from May to June [21].

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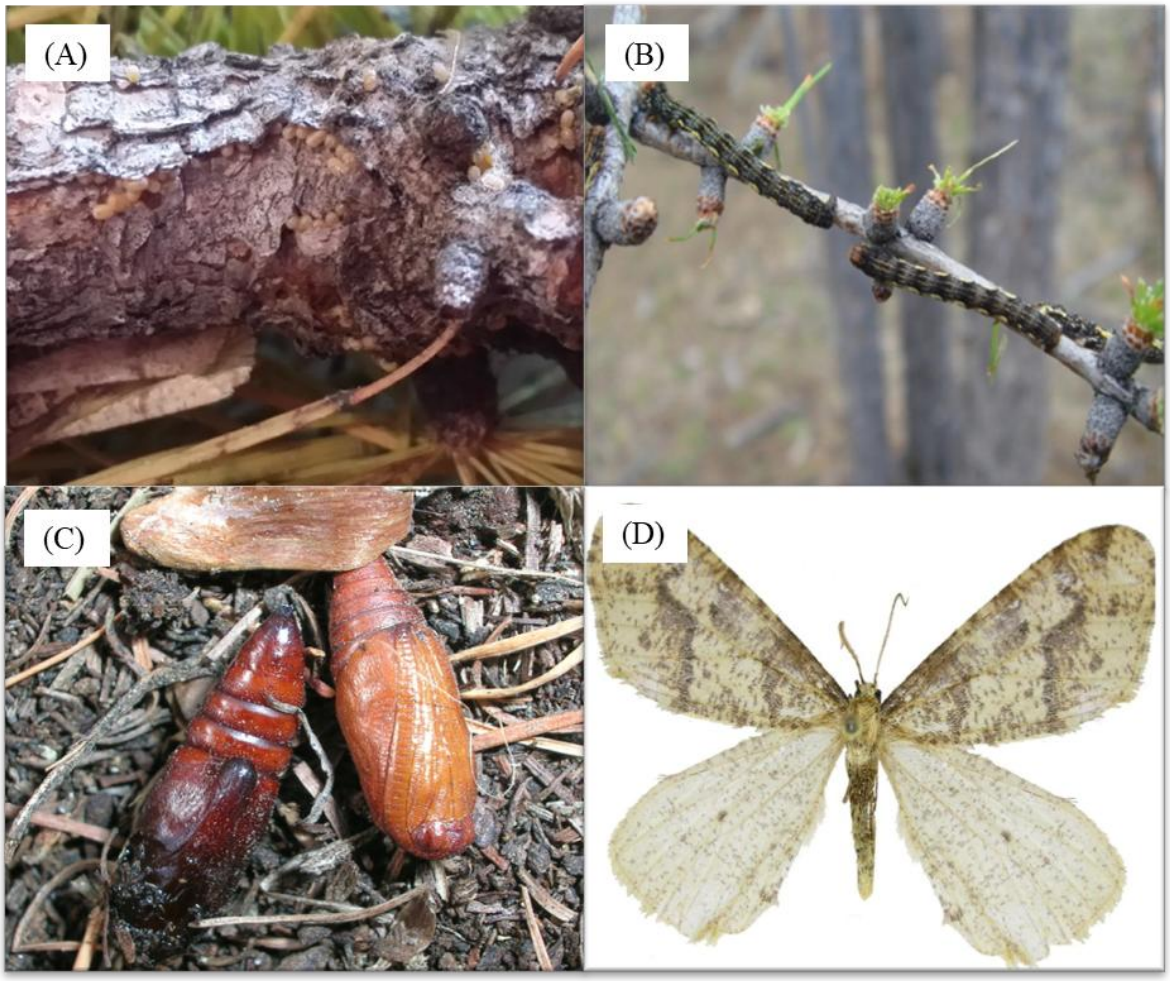

Figure 3. Developmental stages of E.jacobsoni Djak based on morphological characteristics: (A) egg, (B) larva, (C) pupa, and (D) adult.

Laboratory bioassay of bacterial suspension on larvae

After collecting live larval samples, the experiment was conducted using an artificial medium. Disposable food containers were selected, and the lids were perforated with appropriately sized holes to allow air exchange. Fifteen larvae were placed in each container, and 3ml of bacterial suspension was evenly sprayed over them. The containers were then incubated in a thermostat under controlled conditions: a minimum of 8 hours of daily illumination, a temperature range of 23–28°C, and approximately 35% relative humidity. Every two days, the number of larvae was recorded, and the medium (wheat bran) within the containers was regularly replaced. The larvae were resprayed with bacterial suspension each time the medium was renewed [22].

Identification of bacteria

The collected biomass from the overnight-grown bacterial cultures was subjected to genomic DNA isolation using the ZanaSpex Bacterial DNA isolation kit in accordance with the manufacturer's instructions.

The 16S rRNA gene fragments were amplified using universal primers 27F: 5'-ACA GTT TGA TCC TGG CTC AG-3' and 1492R: TGT TAC GAC TT -3' 5'-CGG TTA CCT. PCR mix was prepared with 10xbuffer, 1.5 mM MgCl2, 0.2 mM dNTP, 0.4 µM of each primer, 2.5 IU Taq DNA polymerase (Roche Faststart), and dH20 were added to the PCR mix until the total reaction volume reached 30µl (Dinoto A et al., 2020, Ibrahim., 2016). Parameters of the PCR were first denaturated at 94 °C for 5 minutes; 30 cycles of denaturation at 94 °C for 1 minute,

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annealing at 55 °C for 1 minute, and extension at 72 °C for 2 minutes; followed by a final extension at 72 °C for 5 minutes. PCR products were electrophoresed on a 1.5% agarose gel, and nucleotide sequencing was conducted by Beijing Tsingke Biotechnology Company. To identify the similarity between the obtained 16S rRNA nucleotide sequences and the databases, the NCBI BLAST (Basic Local Alignment Tool) was employed. For the phylogenetic analysis and alignment, MEGA 12 and Snapgene programmes were applied.

Survival analysis (Kaplan–Meier method)

Survival curves were constructed for each treatment group, including control and bacterial treatments, based on time-to-event data. Differences in survival distributions among groups were analysed using the log-rank (Mantel–Cox) test. Statistical significance was set at $p < 0.05$. Median survival time (LT_{50}) and corresponding confidence intervals were calculated for each treatment where applicable. All survival analyses and graphical presentations were performed using GraphPad Prism software (GraphPad Software, USA).

Isolation of *B.thuringiensis* from urban environment

Surveillance and sampling were conducted in forest areas experiencing outbreaks of harmful insects to isolate native bacterial strains with potential insecticidal activity. 56 pure bacterial cultures were successfully isolated and purified from approximately 700 collected samples. These isolates were subjected to preliminary classification and characterisation based on their morphological, physiological, and biochemical properties, and were subsequently prepared for further investigation. Representative *Bacillus* strains were selected from both previously preserved laboratory cultures and newly isolated strains for further analysis.

Results of the bacterial suspension bioassay on larvae

The strain maintained in the laboratory culture collection demonstrated significant insecticidal activity, achieving complete mortality of *E.jaconsoni* larvae within 8 to 10 days. Notably, the newly isolated bacterial culture, designated MNL 78-4, exhibited the highest insecticidal activity, inducing 100% larval mortality within 8 days, which was a statistically significant result ($p< 0.05$).

In one experiment, 15 *E.jacobsoni* larvae were placed in a single experimental container and treated with the MNL 78-4 isolate. After six days, only one larva remained alive, demonstrating a control efficacy six to fifteen times greater than that observed for the other tested strains. These findings highlight the potential of MNL 78-4 as a highly effective biocontrol agent against *E.jacobsoni* under laboratory conditions. Subsequently, the MNL 36 strain inactivated the pest within 10 days, while strains such as MNL 81-3 and MNL 81-4 required 12 days to achieve inactivation. However, among all the isolated strains, MNL Ar-21-1 and MNL Ar-21-2, like the control, failed to inactivate the pest within 14 days.

The results of this study show that native bacterial strains exhibit significant inactivation in their insecticidal activity against *E.jacobsoni* under laboratory conditions. The MNL 78-4 strain, in particular, displayed the fastest and most effective inactivation of *E.jacobsoni* larvae, suggesting its strong potential as a biocontrol agent. This supports earlier research indicating that native *B.thuringiensis* strains are highly effective against Lepidopterapests [14,15]. Given the rising threat of *E.jacobsoni* to Mongolian forests and the absence of focused biological control research in the area, these results offer a vital foundation for future pest management strategies. In a similar study, researchers tested the local strain of *Beauveria bassiana* G07 against EJD larvae in a laboratory.

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They found that at a concentration of 1.5×10^9 spores/ml, the bioinsecticide was highly effective, achieving nearly 92% control over a two week period [23].

Therefore, initiatives like MNL 78-4 can significantly contribute to sustainable forest protection while reducing reliance on chemical pesticides.

Table 2. Survival rate of *E.jacobsoni* larvae treatment with different bacterial strains.

№	Bacterial isolates	Time (days)						
		2	4	6	8	10	12	14
1	Control	15.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	10.0 ± 0.0	8.3 ± 1.25	6.0 ± 0.82
2	MNL 36	15.0 ± 0.0	12.7 ± 0.47	6.7 ± 0.47	0.3 ± 0.47	nd	nd	nd
3	MNL 78-2	14.7 ± 0.47	12.7 ± 0.94	9.0 ± 1.41	7.7 ± 2.05	4.7 ± 1.25	1.0 ± 0.82	nd
4	MNL 78-3	14.3 ± 0.47	13.3 ± 0.47	11.0 ± 0.0	7.3 ± 1.25	5.0 ± 0.0	1.7 ± 1.7	nd
5	MNL 78-4	13.3 ± 0.47	6.7 ± 0.94	1.0 ± 1.41	nd	nd	nd	nd
6	MNL 81-1	14.3 ± 0.47	10.3 ± 1.25	7.0 ± 1.41	3.0 ± 1.41	3.0 ± 1.41	0.7 ± 0.94	nd
7	MNL 81-2	14.3 ± 0.47	12.7 ± 1.89	6.7 ± 1.889	4.7 ± 0.47	3.0 ± 2.16	0.7 ± 0.94	nd
8	MNL 81-3	14.0 ± 0.82	8.3 ± 1.25	3.3 ± 1.70	2.7 ± 1.7	0.7 ± 0.94	nd	nd
9	MNL 81-4	14.7 ± 0.47	13.0 ± 0.82	10.0 ± 0.0	3.3 ± 1.70	0.7 ± 0.47	nd	nd
10	MNL Ap-1-1	14.3 ± 0.47	10.3 ± 1.25	7.0 ± 1.41	3.0 ± 1.41	3.0 ± 1.41	0.7 ± 0.94	nd
11	MNL Ap-21-1	14.7 ± 0.47	12.7 ± 1.89	6.7 ± 1.89	4.7 ± 0.47	3.0 ± 2.16	1.7 ± 1.25	0.3 ± 0.47
12	MNL Ap-21-2	14.3 ± 0.47	12.3 ± 1.70	10.3 ± 1.25	8.7 ± 1.25	8.0 ± 0.82	7.0 ± 1.63	6.3 ± 1.89
13	MNL HA	14.7 ± 0.47	12.7 ± 0.94	9.0 ± 1.41	7.7 ± 2.05	4.7 ± 1.25	1.0 ± 0.82	nd

RESULTS AND DISCUSSION

Results and of survival probability

The results comparing larvae of *E.jacobsoni*. The exposure to *B.thuringiensis* MNL strains in the experimental group was compared to the control group in Figure 4. The Kaplan–Meier survival curves revealed that larvae treated with MNL strains exhibited a rapid decline in survival probability. In contrast, control larvae survived longer with a more gradual decrease in survival. The difference between the two survival curves was statistically significant (p = 0.035), confirming that the MNL strains substantially reduced the survival time of *E. jacobsoni* larvae.

This observation is consistent with the established mechanism of *B. thuringiensis* toxins, particularly Cry and Cyt proteins, which bind to receptors in the insect midgut epithelium, causing pore formation, epithelial lysis, septicemia, and ultimately death [19, 24]. The rapid decrease in larval survival indicates that the strains are not only effective but also act quickly, which helps shorten the time during which larvae can cause damage by feeding.

Our findings align with earlier studies indicating that *B.thuringiensis* strains have potent insecticidal effects against lepidopteran pests.

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Reviews of Cry proteins have highlighted their broad host range and particular effectiveness against Lepidoptera, with successful applications of *B.thuringiensis* formulations in controlling forest defoliators, such as gypsy moth (*Lymantria dispar* (Linnaeus, 1758)). More recently, novel *B. thuringiensis* isolates have been shown to significantly reduce the survival of *Spodoptera litura*, emphasising the value of screening new strains for biocontrol potential. The present study extends this body of evidence by confirming the susceptibility of *E jacobsoni*, a pest of increasing ecological and economic concern, to the MNL strains [25,26,27].

These results demonstrate that this consistency enhances the potential utility of these methods in operational pest management. Moreover, the significant difference between treatment and control survival curves indicates that the effect is not due to random variability, but represents a biologically meaningful impact. On the other hand, from an applied perspective, the ability of MNL strains to rapidly reduce larval survival has important implications for integrated pest management. Fast-acting microbial insecticides reduce crop and forest damage, and their use can mitigate reliance on chemical insecticides. Further studies should focus on characterising the specific Cry proteins or virulence factors present in the MNL strains, as well as evaluating their persistence and efficacy under field conditions. Furthermore, determining their interactions with other natural enemies or microbial agents might uncover synergistic effects that enhance pest control. Such research would contribute to the development of robust, environmentally sound strategies for managing *E.jacobsoni* outbreaks in forestry and agricultural systems.

In summary, the present study provides strong evidence that *B.thuringiensis* strains are highly effective against *E.jacobsoni* larvae, significantly reducing their survival as compared to untreated controls. These findings contribute to the growing body of evidence supporting *B.thuringiensis* as a biocontrol agent and highlight MNL strains as promising options for sustainable pest management strategies.

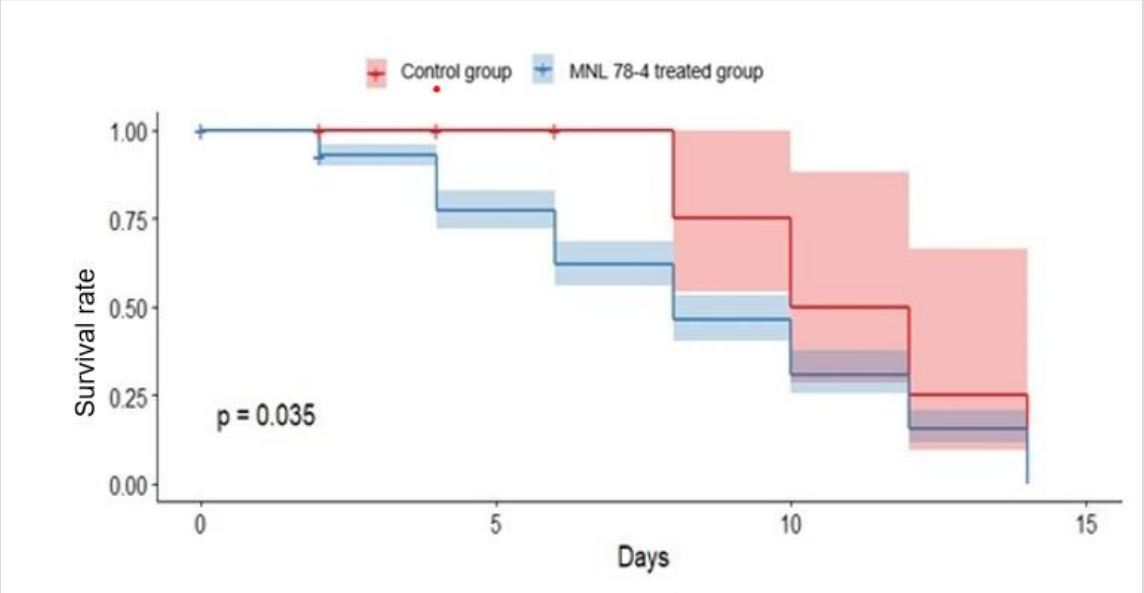

Figure 4. Kaplan–Meier survival curves showing the survival probability of *E.jacobsoni* larvae treated with strain MNL 78-4 compared with the untreated control group.

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Median Lethal Time (LT_{50}) of *E.jacobsoni* Larvae exposed to *different B. thuringiensis* MNL strains

The median lethal time (LT_{50}) values for *E. jacobsoni* Djak. Larvae exposed to different laboratory strains compared with the control are presented in Figure 5. LT_{50} values ranged from as low as 3.8 days to as high as 12.3 days, compared with days in the untreated control group. Strains MNL-78-4 and MNL-36 were the most effective, causing the most rapid mortality with LT_{50} values of 3.8 and 4.3 days, respectively. Several other strains (e.g., MNL-91-3, MNL-81-1, MNL-Ap1.1) exhibited intermediate activity, with LT_{50} values ranging from 5.7 to 6 days. By contrast, some strains (MNL-78-2 and MNL-HA) showed little to no improvement over the control, with LT_{50} values of 12.3 days. These results indicate apparent differences in insecticidal efficacy among MNL strains.

The LT_{50} analysis demonstrates that certain *B.thuringiensis* MNL strains possess strong insecticidal activity against *E. jacobsoni* larvae, while others are comparatively less effective. Strains MNL-78-4 and MNL-36 showed the shortest LT_{50} values, indicating rapid larval mortality and highlighting their potential as highly virulent candidates for biological control. The reduced LT_{50} compared with the control (9.3 days) confirms that these strains significantly accelerate larval death.

Intermediate LT_{50} values (5.7–6.8 days) observed for strains such as MNL-91-3, MNL-81-1, and MNL-Ap1 suggest moderate effectiveness, which could still contribute to pest management when combined with other control strategies. On the other hand, strains with LT_{50} values equal to or higher than the control (MNL-78-2 and MNL-HA) appear ineffective, suggesting possible differences in toxin gene composition, expression levels, or compatibility with the host insect gut environment.

These findings are consistent with earlier studies reporting variability in the virulence of *B.thuringiensis* strains against lepidopteran larvae [17, 25]. Such variation is often linked to differences in Cry protein profiles and host-specific receptor binding. The identification of strains with strong larvicidal activity, such as MNL-78-4 is particularly valuable for integrated pest management (IPM), as they can provide targeted suppression of *E.jacobsoni* populations with reduced reliance on chemical insecticides.

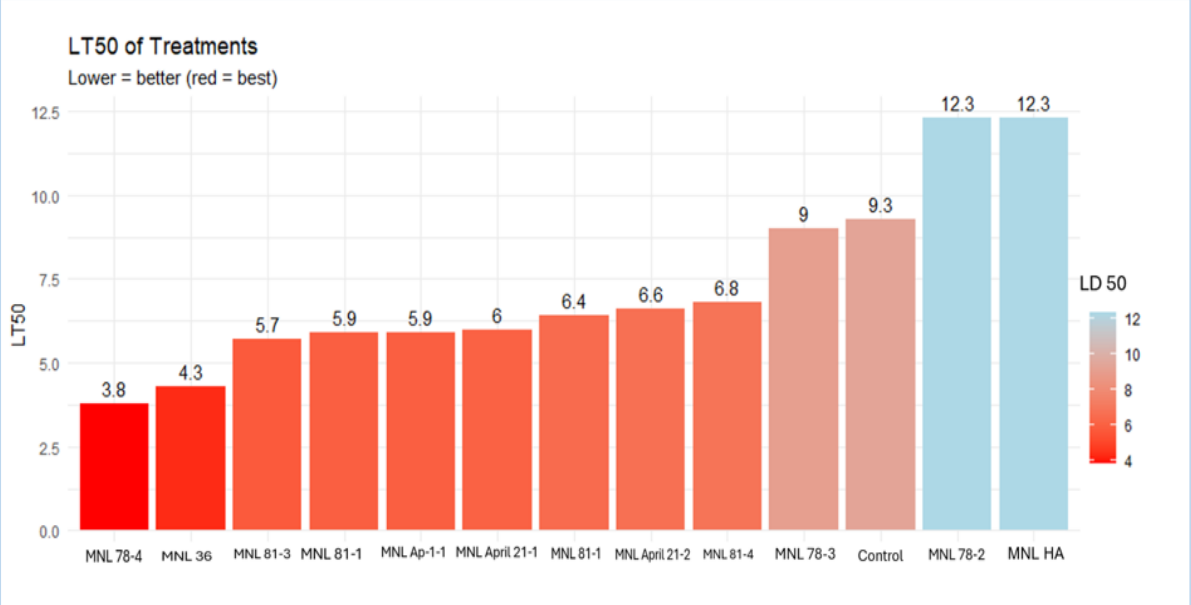

Figure 5. The median lethal time (LT_{50}) values for Erannis jacobsoni Djak. Larvae exposed to different MNL 78-4 strains.

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Overall, the LT_{50} data underscore the importance of strain screening in selecting effective biocontrol agents. While some MNL strains hold substantial promise for pest suppression, others may require further investigation to determine their ecological role or potential synergistic use with other microbial or chemical agents.

Molecular identification of an isolated bacterial strain

PCR amplification of the isolated DNA was conducted using the universal primer pair 27F and 1492R, which target the bacterial 16S rRNA gene region, resulting in a product of approximately 1441 bp. The nucleotide sequence of culture MNL 78-4 was identified using the NCBI GenBank BLAST tool and found to be 99% identical to the reference sequence of *B.thuringiensis MN784218*.

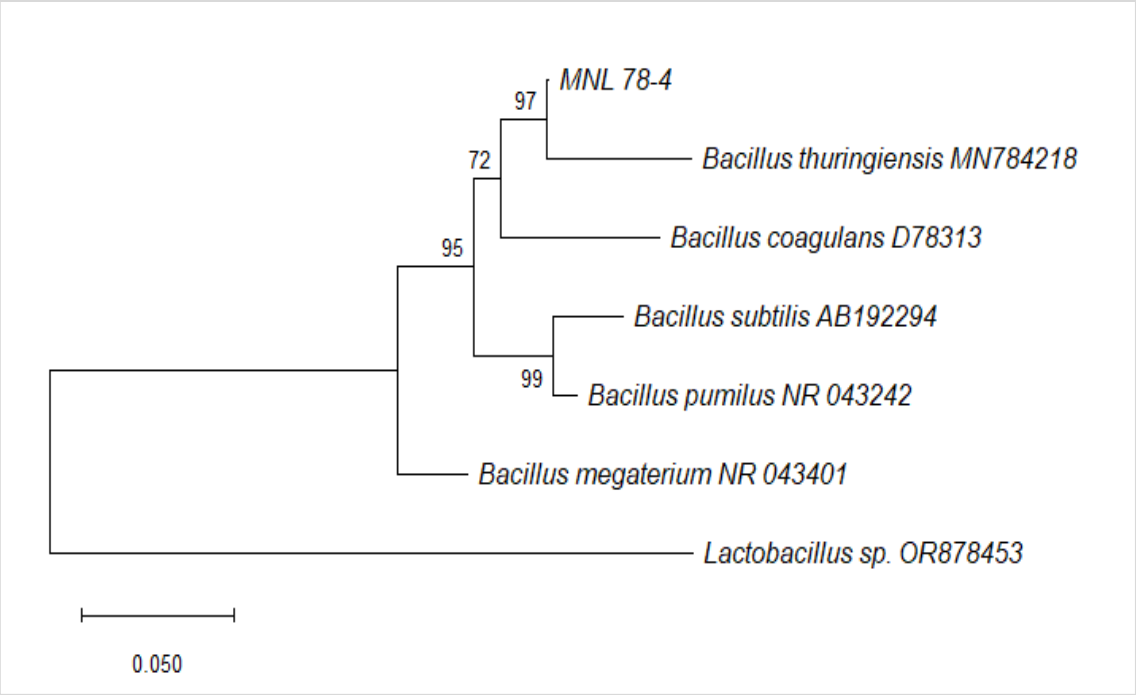

Figure 6. Neighbour-joining phylogenetic tree constructed with 16S rRNA partial sequences of MNL 78-4 and NCBI reference sequences.

The phylogenetic relationships of the taxonomically defined cultures were constructed using the MEGA-12 program. The neighbour-joining method was applied with 1000 bootstrap replicates, and the results are shown in Figure 6.

CONCLUSIONS

The finding of the present study showed that *B.thuringiensis* strain MNL 78-4 is a highly effective biological control agent against Jacobson's moth (*E.jacobsoni* Djak.), which is a major forest pest in Mongolia. Under laboratory conditions, *B.thuringiensis* strain MNL 78-4 showed strong insecticidal activity, resulting in complete larval mortality within 8 days. These results indicate its potential for further development within *B.thuringiensis*-based pest management strategies that can play a crucial role in reducing dependence on chemical pesticides, ultimately promoting ecological balance in the ecosystems.

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Author contribution

The authors confirm contribution to the paper as follows: MeM, EE, ESh, AD, ED,

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GZ, AO, UuM writing original draft, data curation, visualization, and computing. PB, ReTs methodology, review and editing, conceptualisation. EE, ED, ESh, GZ- data curation. MeM, PB, ReTs: writing, review and editing. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare no conflict of interest related to this work.

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