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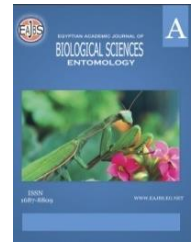
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Intestinal Immune Responses of the Silkworm Larvae, *Bombyx mori* to Bacterial Leaf Blight Caused by *Pantoea ananatis*

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ABSTRACT

The isolation of a novel endophyte *Pantoea ananatis*, a bacterial leaf blight on mulberry leaves is the first record in Egypt. Four bacterial isolates (L1, L2, L4, and L5) were obtained from infected and healthy mulberry leaves and were identified by classical techniques which exhibited short rods and Gram-negative as well as their biochemical and physiological characteristics. Molecular identified rDNA sequences of four isolates were performed. The phylogenetic tree analysis confirmed that all isolates belong to *P. ananatis*. After the pathogenicity test on leaves, bacterial isolates L1 and L4 produced a typical symptom on mulberry leaves. Larvae of mulberry silkworm, *Bombyx mori* were orally infected with each of the four *P. ananatis* isolates separately to assess the silkworms' immune response. Significant variation was observed in the protein concentration and the appearance of the new hemolymph protein bands profile in the infected larvae. It is interesting to note that the immunized hemolymph of the infected larvae exhibited a marked increase in the quantity of antimicrobial peptides with molecular weights less than 30 KDa and the intensity of major proteins. However, the total protein count and humoral immune showed that the bacterial isolates L2 and L5 were more pathogenic to *B. mori*.

INTRODUCTION

Numerous studies have been conducted on *B. mori* because of its economic significance and use as a model insect for Lepidoptera genetics. The only food source for the *Bombyx mori* L., is mulberry leaves (*Morus* sp.), which are crucial for its nutrition, silk production and cocoon formation. Since *B. mori* must be raised indoors, they are susceptible to various infectious diseases caused by bacteria, viruses, fungi, and protozoa (Seidavi *et al.*, 2005; Hiware, 2006). Mulberry is cultivated in Africa, Asia and Europe as

raw materials, mostly for the silk production and medical beauty industries (Yuan and Zhao, 2017). *Pantoea ananatis* was first identified in Guangxi Zhuang, China, from mulberry (*Morus atropurpurea*) roots afflicted with bacterial wilt (Yuan *et al.*, 2023). It is responsible for a variety of disease symptoms in a number of commercial crops, including onions, rice and maize (Asselin *et al.*, 2021; Kini *et al.*, 2020; Capucho *et al.*, 2010). *P. ananatis* is a member of the *Enterobacteriaceae* family, which causes disease in a wide range of monocotyledonous and dicotyledonous species. The increasing number of infections reported on hosts in various parts of the world has led to its classification as a newly discovered pathogen. The expansion of geographical areas was first observed in the Philippines, Japan, Korea and Italy (Tabei *et al.*, 1988, Kim *et al.*, 1989, Serrano, 1928, Cortesi and Pizzatti, 2007). *P. ananatis* is a common pathogen, endophyte, symbiont, saprophyte and epiphyte that inhabits a wide range of ecological niches. Even human bacteremia has also been reported to be caused by *P. ananatis* (De Baere *et al.*, 2004).

In nature, an insect's gut is constantly infected with various pathogens, consequently, it's critical to understand the insect gut immune system's reaction to the invading bacteria. *B. mori* can become infected with a wide variety of bacteria, such as *Staphylococcus* sp, *Bacillus* sp, *Proteus* sp, *Serratia* sp, *Klebsiella* sp, *Enterococcus* sp, and *Pseudomonas* sp (Watanabe *et al.*, 1998; Jin and Lu, 2001; Ma *et al.*, 2019; Mohanta *et al.*, 2015; Zhang *et al.*, 2020). In order to combat bacterial infections, *B. mori* has evolved a number of delicate defense mechanisms that combine the use of physical barriers and innate immune systems (Ma *et al.*, 2021). The first line of defense against different bacteria is the physical barriers such as hard body walls made of wax and chitin and peritrophic membrane in the intestine (Kuraishi *et al.*, 2011). The majority of bacterial infections are prevented by the physical barrier; when bacteria get past it, silkworms trigger the innate immune system to stop the pathogen from growing (Hegedus *et al.*, 2009). Cellular immunity and humoral immunity are the two subtypes of silkworm innate immunity. Encapsulation, phagocytosis and nodulation are examples of cellular reactions known as blood cell (hemocyte)-mediated responses (Hoffmann, 2003). The generation of reactive oxygen species (ROS), the prophenoloxidase (PPO) cascade to combat bacterial infection and the activation of the Toll and Imd pathways to generate antimicrobial peptides (AMPs) are the important mechanisms involved in humoral immunity (Jang *et al.*, 2021). The aim of this study is to ascertain how the gut immune system of insects responds to oral infection with the Gram-negative (*P. ananatis*) bacteria and how this response affects the overall hemolymph immune proteins (AMPs) of *B. mori* in terms of both quantitative and qualitative profiles.

MATERIALS AND METHODS

Mulberry Samples Collection, Isolation, and Pathogenicity Test of Endophyte Bacteria:

Healthy and infected leaves from mulberry cultivars (*Morus alba* L.) were collected from the Alexandria governorate. For isolation, surface sterilization of the mulberry then using the maceration technique (Liotti *et al.*, 2018). Shortly, 1 g of disinfested leaf tissue macerate was serially diluted in 0.8% NaCl and then plated on nutrient agar (N.A) medium plates (Coutinho & Venter, 2009). For purification using Yeast extract–dextrose–calcium carbonate agar (YDC) (Azad *et al.*, 2000) and PA 20 medium, semi-selective media (Goszczyńska *et al.*, 2006; Ashmawy *et al.*, 2020a). The plates were incubated at 29°C for 48 hrs. Then, colonies with different morphological characteristics were purified.

Pathogenicity testing was performed for all bacterial isolates, whether from healthy tissues or leaves infected with mulberry bacterial blight. Use the detached leaf method. After sterilizing the surfaces of both infected and healthy leaves for five minutes with a 2% sodium hypochlorite solution, the leaves were rinsed with sterile distilled water and allowed to air dry. Each purified bacterial isolate was suspended in a sterile 0.2 M phosphate buffer. The suspension was adjusted to standard inoculum [10^{-9} CFU/ml⁻¹] at 620 nm. The leaves were put in humidity plates or a chamber after soaked in bacterial suspension, the plates were incubated after being sealed with Parafilm. The mulberry control leaves were soaked in a sterile phosphate solution. Each leaf was inoculated at a temperature of $29 \pm 2^\circ\text{C}$ until symptoms appeared. This examination was duplicated three times. (Sharafati *et al.*, 2014).

Identified by Biochemical and Morphological Tests:

Morphological characteristics of bacterial colonies were directly examined using light microscopy and Gram test (3% KOH). While physiological characteristics of isolates include catalase activity, starch hydrolysis, carbon utilization (maltose, sucrose, mannitol, and glucose), and the Indole test uses Kovac's method. All tests were repeated at least twice and determined by standard methods (Schaad *et al.*, 2001; Aneja, 2003).

Molecular Identification:

DNA Extraction:

The total genomic DNA of the isolate was extracted using a mini-preparation DNA isolation technique according to Nurjanh *et al.* (2017). As much of 1.5 ml of liquid L.B media with bacterial cell growth overnight were centrifuged for two minutes at 5,000 g. After the DNA pellet was diluted with 540 μl of TE buffer (0.1 M Tris-HCl, 0.1 M EDTA pH 8), 30 μl of 10% SDS was added, and the mixture was incubated for 60 minutes at 37°C . Then the pellet was mixed with 100 μL of 5 M NaCl and 80 μL of CTAB/NaCl. It was incubated for 10 minutes at 65°C before 700 μl of chloroform isoamyl alcohol (24:1) was added, and it was centrifuged for 5 minutes at 12,000 g. The top layer was put into an Eppendorf tube measuring 1.5 mL. After adding 600 μL of isopropanol, the mixture was centrifuged for five minutes at 12,000 g. After the pellet was air-dried and washed with 70% ethanol, it was diluted with 20 μL of TE buffer.

PCR Amplification:

PCR amplification of the 16S rRNA gene was performed using the isolates' extracted DNA. Using the universal 16S rRNA gene primers F (5'GAAGAGTTTGATCC TGGCTCAG3') and R (5'CTACGGCTACCTTGTGTTACGA3'), this gene was amplified according to Ausubel *et al.* (1992) and Srinivasan *et al.* (2015). The reaction was performed in a 50 μL total volume that contained 5 μL 10 x buffer, 2 μL 10 pmol of forward primer and reverse primer, 4 μL 25 mM MgCl₂, 4 μL 2.5 mM dNTPs, 2 μL 50 ng of bacterial genomic DNA and 0.4 μL (5 units/ μL) Taq DNA polymerase - Promega, Germany. Using a thermal cycler (Techne, UK), PCR amplification was carried out for one cycle of 95°C for five minutes, then 34 cycles of 45 s at 95°C for denaturation, one min at 50°C for annealing, and 2 min at 72°C for elongation. For the last extension, the reaction mixture was then incubated for 10 minutes at 72°C (Ashmawy *et al.*, 2020b).

16S rRNA Sequencing and Analysis:

The amplified product (1550 1600 bp) of 16S rRNA. CentriSep spin columns were used to purify sequencing products before isolates were delivered to (Scientific Services Company, lab Technology) for sequencing. Utilizing the getting sequences of the amplified regions, the identification of bacterial isolate was validated by utilizing the Basic Local Alignment Search Tool (BLAST search) on the National Centre for Biotechnology Information (NCBI) site (<https://www.ncbi.nlm.nih.gov>). Molecular Evolutionary Genetics Analysis (MEGA X) software was used to align a few reference strains with

similar GenBank isolates. MEGA X software was used to create a phylogenetic tree using the neighbor-joining (NJ) approach (Kumar *et al.*, 2018). The sequences that were acquired were compared to several worldwide bacterial isolates that were sourced from GenBank.

Insect Rearing:

The local hybrid silkworm eggs were provided by the Sericulture Research Department, Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt. In a lab setting at 25°C, 75% relative humidity, and a 12-hour light–12-hour dark photoperiod. Four times a day, new mulberry leaves were fed to *B. mori* larvae.

Oral Infection and Hemolymph Collection:

The four *P. ananatis* isolates (L1, L2, L4, and L5) were cultivated in N.A broth medium at 29 °C overnight. To trigger the immune responses, the silkworm larvae were fed on mulberry leaves, which were cut into 2×2 cm pieces and coated with the bacterial isolates suspension (10^8 /larva), before being incubated at 25°C. The oral infection was conducted on three groups of fifty larvae each. A control of 0.85% NaCl or 10 µL of bacteria was fed to each larva. Twenty-four hours after the bacteria were infected, hemolymph was collected and centrifuged at 10,000 g for ten minutes to remove hemocytes into sterile tubes along with a few phenylthiourea crystals. A total of ten larvae's cell-free hemolymph samples were either used immediately or stored at -20 °C until needed.

Total Hemolymph Protein (THP) Determination:

Bradford's method (Bradford, 1976) was used to calculate the protein concentrations in hemolymph samples. A standard curve for protein estimation was created using dilutions of bovine serum albumin (BSA).

SDS Electrophoresis:

According to Laemmli (1970), a discontinuous buffer system was used for the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) procedure. The sample buffer (2% SDS, 0.0625M Tris-HCl, pH 6.8, 0.002% bromophenol blue, 10% glycerol, with 5% β-mercaptoethanol) was combined with an equal volume of the hemolymph sample. The mixture was then centrifuged at 10,000g for five minutes at 50°C after heat treatment. After being electrophoresed, 10% T and 4.5% T gels were used for separation and stacking, respectively, (Coomassie blue R-250) 0.1% (Bio-Rad) was used to stain the gels. Using a standard protein marker (Bio-Rad), the molecular weights (MW) of the separated proteins were determined. The Total Lab Software Analysis (Microsoft version 11.0) was used to quantitatively determine the resolved protein bands.

RESULTS

Isolation and Pathogenicity of Associated Bacteria with Mulberry Leaves:

Results revealed that (L2 and L5) bacteria were isolated from healthy leaves. Also, isolates (L1 and L4) of bacteria were isolated from infected leaves and showed natural leaf blight symptoms (Fig. 1). Isolated bacterial colonies produced yellow pigment on the (N.A. medium), and were convex, whole, smooth, viscid, and circular in shape. Bacterial isolates cultured on YDC media also produced yellow colonies, however on AP20 medium, they created shining, yellow drop-shaped colonies (Fig. 2).

All the bacterial isolates were tested for the pathogenicity test of healthy and infected mulberry leaves. Symptoms appear as chlorosis spread throughout leaves with (L1 and L4) *P. ananatis* isolates. Endophyte isolates (L2 and L5) show that no leaf blight symptoms were recorded as well as the control leaves.



Naturally infected of bacterial leaf blight
Fig 1. Natural bacterial leaf blight on mulberry leaves

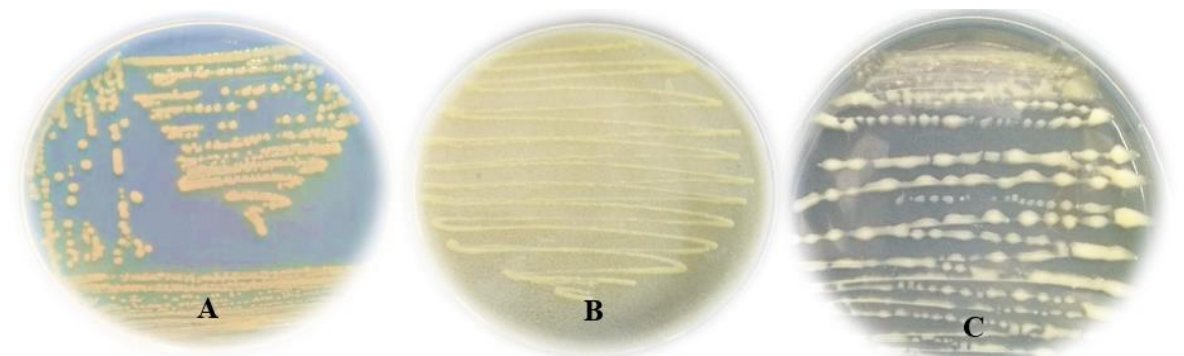


Fig 2. Isolation and purification on (A) nutrient agar (N.A) medium, (B)Yeast extract-dextrose-calcium carbonate (YDC) medium and (C) AP20 medium.

Identified *P. ananatis* by Biochemical and Physiological Characteristics:

All the investigated bacterial isolates were short rod, Gram-negative, and non-spore producing, according to the data in (Table 1). The isolated bacteria reacted positively to 3% KOH, grew on 4% NaCl, and did not produce any fluorescent pigment on the KB medium, were positive for catalase and indole but negative for starch hydrolysis. However, the production of acid from mannitol, glucose, maltose, and sucrose was detected in all isolates.

Table1. Biochemical and physiological characteristics of all bacterial isolates from mulberry associated with natural bacterial blight leaf symptom.

Characteristics														
Shape (rods)	3% KOH	Motility	aerobic growth	Sporulation	Fluorescent pigment on KB medium	Growth at 36°C	catalase activity	Production of indole	starch hydrolysis	Growth in 4% NaCl	Acid production from:			
											Glucose	Maltose	Mannitol	Sucrose
+	+	+	+	-	-	+	+	+	-	+	+	+	-	+

”+” = positive reaction, ”-” = negative reaction

Molecular Identification:

PCR Amplification, 16S rRNA Sequencing and Analysis:

Total genomic DNA was extracted and used as a template to amplify the partial 16S rRNA gene using a universal primer. Moreover, the 16S rDNA sequences of four isolates (L1, L2, L4, and L5) were placed on the NCBI Gene Bank website's nucleotide sequence database: <https://www.ncbi.nlm.nih.gov/>. The search revealed that the sequence corresponding to the associated bacterial associated mulberry blight leaf is identical (99.93 - 100% homology) to *Pantoea ananatis* (accessed on:7, Aug 2022) under accession numbers OP108829, OP108830, OP108831, and OP108832 respectively.

Data presented in (Fig. 3) showed phylogenetic tree based on the neighbor-joining method of four isolates constituted a two cluster with *P. ananatis* and other GenBank deposited *Pantoea* spp. Strains. Hence, it was confirmed that all isolates belong to *Pantoea ananatis*.

Impact of Bacterial Infection on The Total Hemolymph Protein Content:

A comparison of groups of infected and uninfected larvae reveals the total protein concentration of the hemolymph (Fig. 4). Compared to the infected groups, the mean value obtained from the control was significantly higher, measuring 36.57 mg/ml. The infection of larvae with the isolates (L2 and L5) resulted in the lowest protein content (22.93 mg/ml and 25.5 mg/ml, respectively). Infected larvae from the L1 isolate had a mean protein concentration of 28.47 mg/ml, whereas the L4 isolate was 26.87 mg/ml. However, the average protein concentration in the infected larvae isolates is still lower than that of the control.

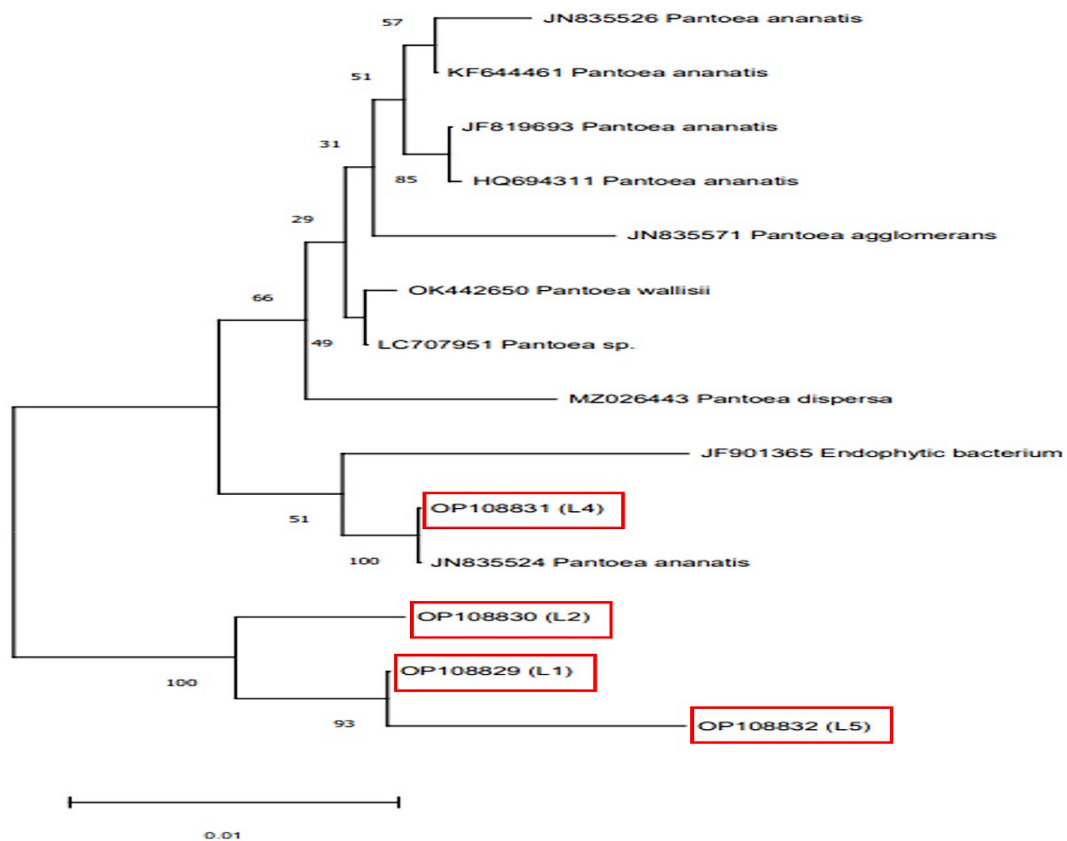


Fig 3. Neighbour-joining dendrogram of the partial, GenBank derived of 16S rRNA gene sequences between isolates L1, L2, L4, L5 and other *Pantoea* species.

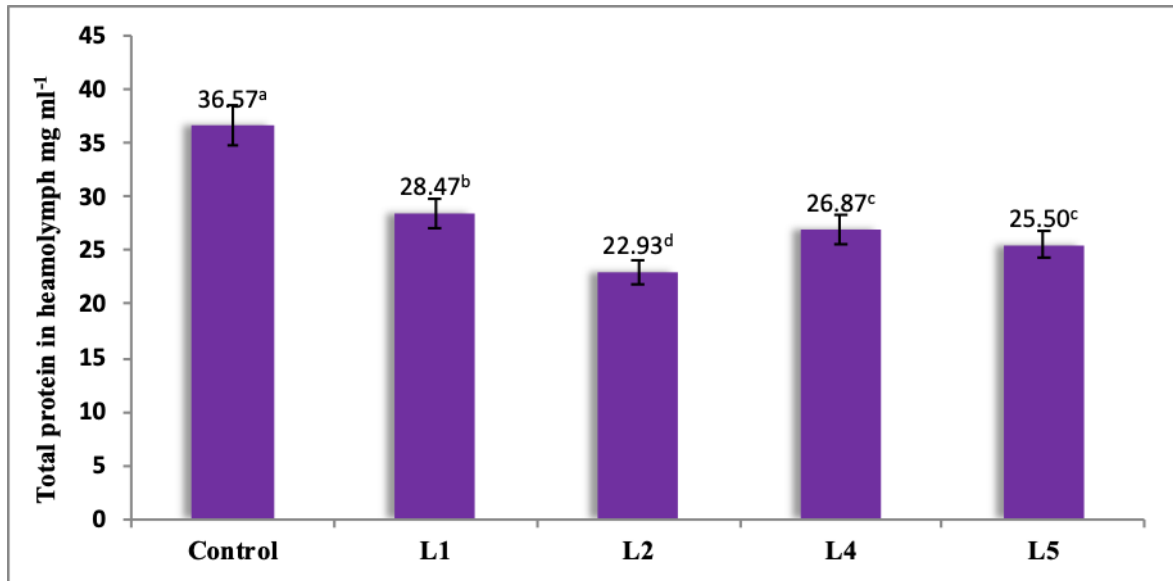


Fig 4. Protein content of the total hemolymph in *B. mori* larvae of non-infected and infected with bacterial isolates L1, L2, L4, and L5, respectively. The bars represent mean values \pm SE.

Hemolymph Protein Electrophoretic Patterns of Infected and Non-Infected Larvae:

The hemolymph protein profile of *B. mori* fifth instar larvae was analyzed using SDS-PAGE, wherein the lane was loaded with alternating fractions of control and infected hemolymph 24 hours post-bacterial infection (Fig. 5). The electrophoretic profile identified seven major bands of proteins in the hemolymph with molecular weights of ~ 300 , ~ 80 , ~ 75 , ~ 70 , ~ 30 , ~ 22 , and ~ 4 kDa; they had molecular weights between ~ 4 to ~ 300 kDa. In both control and infected larvae, the main polypeptide bands (70–80 kDa) were better characterised. A total of 11 subunit bands with molecular weight ranging between ~ 4 to ~ 300 kDa were detected in the control. Patterns revealed the existence of 16 protein bands at the (L1) isolate, including new bands with molecular weights of approximately ~ 100 , ~ 120 , ~ 130 , ~ 140 , and ~ 200 kDa. The molecular mass of ~ 4 kDa was better characterized in the control over the (L1) isolate. The isolates (L2, L4, and L5) showed an additional increase in the number of haemolymph protein subunit bands. Twenty subunit bands were identified, with new corresponding molecular masses of ~ 7 , ~ 10 , ~ 14 , and ~ 27 kDa. The results demonstrate a rapid increase in the haemolymph protein composition and concentration of the (L2 and L5) isolates, the intensity of the major proteins was significantly higher, especially ~ 4 , ~ 70 , ~ 75 , ~ 80 , and ~ 300 kDa.

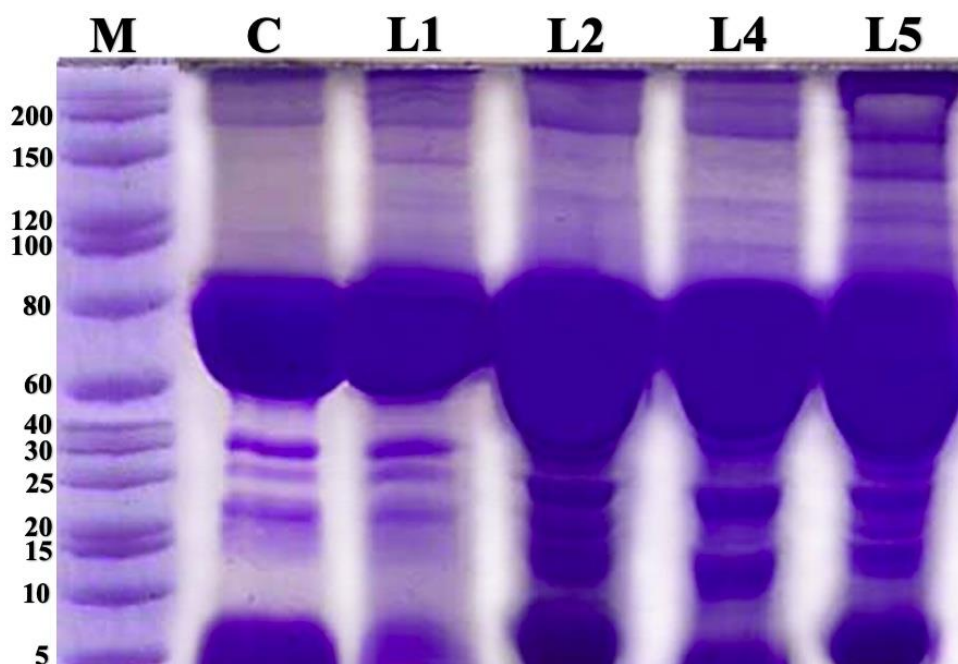


Fig 5. 10% SDS-PAGE electrophoretic protein profile of *B. mori* hemolymph; lane 1: (M) standard protein marker, lane 2: (C) control, lane 3: the hemolymph of (L1) isolate, lane 4: the hemolymph of (L2) isolate, lane 5; the hemolymph of (L4) isolate and lane 6: the hemolymph of (L5) isolate. Samples containing 10 µg of total protein were added to each lane.

DISCUSSION

Based on the collected data and those provided by Nurjanh *et al.* (2017), the tested isolates were identified based on their morphological and biochemical features were in conformity. Additionally, molecular approaches such as 16S rRNA fragment amplification have more recently become important to identify an unknown bacterium to the genus or species level. According to data from the first report of *P. ananatis* producing leaf blight on strawberries in Egypt by Abdel-Gaied *et al.* (2022), the causal pathogen of leaf blight was identified as *P. ananatis*. The genus *Pantoea* is categorized under the *Enterobacteriaceae* family (Gavini *et al.*, 1989), which has been isolated from humans, animals, and a variety of biological niches, such as plants, water, and soil (Walton and Stavrinos, 2015). Some species have been identified as plant pathogens, while others are opportunistic human infections. They are commonly found in association with plants as endophytes or epiphytes (De Baere *et al.* 2004). Endophytes are microorganisms that reside inside plants without harming them, they help their hosts obtain nutrients and suppress diseases in some species. On the other hand, some bacterial endophytic isolates from healthy plants impeded some seedlings' growth in reinoculation assays, by producing specific metabolites (Van Peer *et al.*, 1990). Our current study indicated that *P. ananatis* as endophytic bacterial isolates which were isolated from internal tissues of mulberry healthy leaves can't show typical blight symptoms. However, it has also been noted that *P. ananatis*, which causes mulberry leaves to exhibit typical symptoms of bacterial leaf blight disease, can elicit disease symptoms in a variety of hosts. Numerous researchers have looked into the pathogenicity of *Pantoea* in a variety of plant hosts and have found potential causes of pathogenicity over the course of disease development (Abdel-Gaied *et al.*, 2022).

***B. mori* Immune Response:**

The key to preventing diseases in silkworms is to maintain cleanliness and hygiene throughout the rearing process. Diseases can affect any larval instar, but they most frequently strike the last larval instar, which results in losses to the cocoon yield (Steinhuaus, 1949). The current finding indicates a remarkable reduction in the total protein concentrations (28.47 mg/ml, 22.93 mg/ml, 26.87 mg/ml, and 25.5 mg/ml) upon infection of the fifth instar larvae with bacterial isolates L1, L2, L4, and L5, respectively. Numerous studies have documented the protein concentrations in the *B. mori* hemolymph; in both sexes, these concentrations began at a low level at the beginning of the final larval instar and increased gradually until they peaked prior to pupation (Mahmoud, 1973; Fujiwara and Yamashita, 1998; Shahin *et al.*, 2013). The average protein concentration in the infected larvae in this work is still lower than that of the control. The same results of THPs of *Spodoptera littoralis* were observed when *Staphylococcus* was injected into the larval stage. This is likely due to the bacteria's extensive consumption of plasma proteins during growth and multiplication (Radwan *et al.*, 2022).

The hemolymph protein profile of *B. mori* larvae in this study revealed a noteworthy subunit difference between the infected and control larvae. In contrast to the proteins observed in normal larvae, the synthesis of a novel protein with a molecular weight of (200–100) kDa and a lower 30 kDa was observed. The highest protein bands and intensity were detected when larvae were infected with (L2 and L5) followed by (L4 and L1) isolates, respectively, however, still higher than that of the control. Numerous proteins linked to immunity and nutrient storage have been found in *B. mori*'s hemolymph such as storage proteins, apolipoproteins and 30K proteins (Zhang *et al.*, 2014). It has been suggested that 30K proteins are involved in the immune response of silkworms. After injecting *Escherichia coli* into fifth instar larvae, the expression patterns of 30 K proteins were markedly upregulated, suggesting their role in the immune response (Ye *et al.*, 2021). The antimicrobial peptides (AMPs) released into the hemolymph as the final product of immune pathways; they have a low molecular weight ≤ 30 kDa and fight a wide range of microbes with their broad-spectrum antimicrobial activity. Six families of AMPs have been found and documented in silkworms: cecropin, attacin, defensin, moricin, lebocin and gloverin (Tanaka *et al.*, 2008). Moricin was first isolated from the *B. mori* haemolymph, it has 42 amino acid residues and is more effective against Gram-positive than Gram-negative bacteria (Hara and Yamakawa 1995). Four genes have been identified as Bmglv1, Bmglv2, Bmglv3, and Bmglv4 that encode gloverins, which are glycine-rich peptides with a molecular weight of (30-8) kDa in *B. mori*. Bmglv2 increased the permeability of two Gram-negative bacteria cell membranes and inhibited their growth (Kaneko *et al.*, 2007). At very low concentrations, cecropins exhibit antibacterial action against a range of Gram-positive and Gram-negative bacteria (Cheng *et al.*, 2006). Attacins have molecular weight (23-20 kDa) and prevent the synthesis of proteins that make up the outer cell membrane of bacteria (Engstrom *et al.*, 1984). The four protein-encoding genes that make up the lebocin family are Leb1, Leb2, Leb3, and Leb4 and demonstrated to possess antimicrobial activity against fungi, Gram-positive and negative bacteria (Rao *et al.*, 2012). Defensins, which have a molecular weight of 4 kDa, work by disrupting the bacterial membranes to prevent their growth (Lehrer *et al.*, 1989). Even though the larvae infected with the isolates (L2 and L5) had the lowest protein content, there was a noticeable increase in the protein composition in their SDS hemolymph profile. This could indicate a relationship between

AMP synthesis and bacterial invasion because of their differing abilities to thwart silkworm immune responses. Our findings are consistent with a prior study that discovered that oral infection with Gram-positive (*Staphylococcus aureus*) and -negative (*Escherichia coli*) bacteria resulted in an up-regulation of AMPs gene expression in the midgut and hindgut of *B. mori* intestinal tissue. (Riddell *et al.*, 2009).

Conclusion:

The purpose of this work was to identify a pathogenic bacterial strain from diseased mulberry leaves and assess the pathogenicity of *Pantoea ananatis* on a mulberry silkworm, *Bombyx mori*. The study of *B. mori* immune systems has received a lot of attention since infectious diseases in silkworms have the potential to cause a reduction in sericulture and economic losses. Our results provide a novel perspective on the role of *P. ananatis* as a new pathogen to *B. mori*, and show how the introduction of *P. ananatis* to healthy silkworms triggers the production of hemolymph antimicrobial peptides (AMPs). By lowering the total protein content of the infected larvae, we hypothesised that the bacterial isolates L2 and L5 were more harmful to *B. mori*, and more research is necessary to determine the death rate, symptoms, and cure of the infected larvae.

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors have no competing interests to declare that are relevant to the content of this article.

Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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ARABIC SUMMARY

الاستجابات المناعية المعوية ليرقات دودة القز للإصابة ببكتريا *Pantoea ananatis* المتسببة لمرض لفحة الأوراق البكتيرية

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تم عزل البكتريا الداخلية *Pantoea ananatis* من أوراق التوت المصابة بمرض لفحة الأوراق البكتيرية، حيث يعد أول تسجيل لها في مصر بهذه الإصابة على أوراق التوت. تم الحصول على أربع عزلات بكتيرية (L1, L2, L4, L5) وذلك من أوراق التوت المصابة والسليمة، وقد تم تشخيص العزلات البكتيرية بالتقنيات الكلاسيكية التي أظهرتها كعصيات قصيرة، سالبة لصبغة جرام، وغير مكونة للجراثيم بلاضافة إلى خصائصها البيوكيميائية والفسولوجية. تم التعريف بالطرق الجزيئية بإجراء تفاعل البلمرة المتسلسل PCR للحمض النووي الريبوسومي 16SrDNA للاربع عزلات وأكد تحليل الشجرة الوراثية أن جميع العزلات تنتمي إلى *Pantoea ananatis* وبعد اختبار القدرة الامراضية للعزلات البكتيرية على أوراق التوت، اظهرت العزلات البكتيرية L1 و L5 فقط نفس الاعراض النموذجية لمرض لفحة الاوراق على نباتات التوت. تم إصابة يرقات دودة القز التوتية *Bombyx mori* عن طريق الفم بكل من عزلات *Pantoea ananatis* الاربعة السابقة بشكل منفصل من أجل تقييم الاستجابة المناعية لدودة القز. ولوحظ وجود تباين كبير في تركيز البروتين الكلي وكذلك ظهور بروتينات جديدة في دم اليرقات المصابة. ومن المثير للاهتمام أن دم لليرقات المصابة أظهر زيادة ملحوظة في كمية البيبتيدات المضادة للميكروبات ذات الاوزان الجزيئية أقل من 30 كيلو دالتون وفي كثافة البروتينات الاساسية المميزة ليرقة دودة القز. حيث لوحظ أن تركيز البروتين الكلي والمناعة الخلطية في دم اليرقات المصابة بالعزلات البكتيرية L5 و L2 كانت أكثر إمراضية ليرقات دودة القز.