FACULTY OF CIVIL ENGINEERING & TECHNOLOGY FKTA
Universiti Malaysia Perlis (UniMAP)
FKTA Postgraduate Colloquium 2025
UniMAP UNIVERSITI MALAYSIA PERLIS

Comparison of *Bacillus thuringiensis israelensis* Spore Growth Using Different Fruit-Based Waste Media

Nurul Afiqah Japri[1], Hazirah Pengiran[1,*], Amin Rukaini Mustafa[2]

[1]Faculty of Civil Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Perlis, Malaysia
[2]Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP), Perlis, Malaysia

KEYWORDS

Bacillus thuringiensis israelensis
Spore production
Fruit waste
Alternative culture media
Mosquito control
Sustainable biocontrol

ABSTRACT

Mosquito-borne infections remain a global health issue and necessitate cost-effective and environmentally safe vector control methods. *Bacillus thuringiensis israelensis* (Bti) has emerged as an effective microbial larvicide; however, mass production has been hindered due to the cost of conventional growth media. Here, we consider the possibility of utilizing three fruit wastes (watermelon rind, banana peel and citrus peel) as low-cost alternative substrates to grow Bti spores. Fruit wastes were dried, hydrolyzed and filtered, adjusted to approximately pH 7 and sterilized as a method of generating culture media prior to inoculation with Bti. Growth of spores was studied through growth curve analysis, viable spore count and validated under a microscope. Among substrates tested, banana peel extract showed the highest supporting absorbance values and spore viable count at 4.39 $\log_{10}$ CFU/mL, which indicate highest bacterial proliferation and spore formation. Although the fruit-based waste media achieved approximately half the growth performance of the standard medium, it still effectively supported Bti proliferation. These results hold promise of using food wastes in microbial culture and are the baseline of further optimization and up-scaling of producing Bti as a larvicide.

1. INTRODUCTION

Mosquito-borne diseases, such as dengue fever, malaria, and Zika virus infection, are still significant global public health problems. Biocontrol techniques are also considered more as alternatives to chemical insecticides since chemical insecticides are plagued with problems of environmental toxicity and insecticide resistance. Among biological control agents, *Bacillus thuringiensis israelensis* (Bti) has proven to be a highly effective microbial larvicide as it produces spores and parasporal crystal proteins (Cry and Cyt) with high specificity against mosquito larvae (Valtierra-de-Luis et al., 2020).

However, industrially scalable production of Bti remains expensive due to the use of conventional culture medium such as Nutrient Broth (NB) and Tryptic Soy Broth (TSB). Therefore, discovering potential low-cost and locally obtained substrates is essential for enhancing the efficiency and economic viability of Bti manufacturing. Food wastes are good prospects in this regard since they are produced voluminously and almost entirely wasted at low or negative economic costs and carry diverse nutrients that can support microbial growth. Through the utilization of food waste as an alternative culture medium for Bti, this approach helps promote waste valorization, reduce production costs, and subsequently support sustainable mosquito control.

In this study, locally available fruit wastages (watermelon rind, banana peel and citrus peel) were screened as potential substrate sources for Bti cultivation. Growth of Bti as well as spore formation were determined from growth curve profiling, spore viability counts and microscopic examination, respectively. This investigation intends to determine the optimal fruit-based waste medium that may support elevated bacterial proliferation and spore generation, thereby offering a cost-effective and sustainable method for large-scale production of Bti biolarvicide.

2. EXPERIMENTAL PROCEDURE

2.1 Inoculum Preparation

Bti spores were heat-treated at 80 °C for 15 min to eliminate contaminants while maintaining viability, then streaked onto Tryptic Soy Agar (TSA) and incubated at 30 °C overnight. A single colony was used to establish a starter culture in 5 mL of broth, incubated at 30 °C, 180 rpm for 12–18 h. For the main culture, 1 mL of starter was inoculated into 100 mL of food waste-based broth (1:100, v/v) and incubated at 30 °C, 180 rpm for up to 6 days.

2.2 Preparation of Food Waste Media

Fruit wastes were dried overnight, ground, sieved and hydrolyzed in distilled water at 80–95 °C for 1 h. All substrates then were filtered, adjusted to pH ≈ 7, and sterilized by autoclaving before inoculation.

*Corresponding author: hazirahpengiran@unimap.edu.my

2.3 Growth Monitoring

Bacterial growth was assessed by measuring optical density at 600 nm (OD_{600}) using a UV–Vis spectrophotometer, with uninoculated medium as blank. Samples were taken at 2-3 h intervals during the first 3 days (log phase) and every 6–8 h thereafter until day 5. Growth curves were constructed to represent bacterial proliferation and spore formation.

2.4 Spore Quantification

Spore viability was assessed by serial dilution and drop-plate counting. On final day of incubation, cultures were serially diluted (10^{-1}–10^{-6}) in sterile distilled water, and 10 µL aliquots were plated in triplicate on TSA. Plates were incubated at 30 °C for 14–16 h. The viable cell count ($CFU \cdot mL^{-1}$) was calculated using the formula:

CFU/mL = (Number of colonies × Dilution factor) / Volume plated (mL)

2.5 Microscopy

Spore formation was examined using the Schaeffer–Fulton staining method. Briefly, heat-fixed smears were stained with malachite green by steaming for 6 min, rinsed, and counterstained with safranin O for 30 sec. Slides were observed under 100× oil immersion, and representative images were captured.

3. RESULTS AND DISCUSSION

3.1 Growth of Bti in Different Fruit-based Waste Media

Growth of Bti spores was studied with a growth curve analysis, which was then verified using a microscope to confirm spore formation. The growth pattern of Bti on fruit-based waste media were compared with a commercial-quality Tryptic Soy Broth (TSB), as shown in Figure 1.

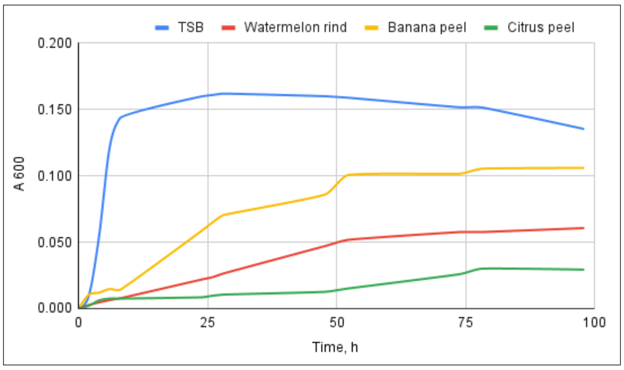

Figure 1. Growth curve of *Bacillus thuringiensis israelensis* in different media (TSB, watermelon rind, banana peel and citrus peel) measured at 600 nm.

The conventional TSB medium was used as a standard control. Absorbance readings at A600 represent the rate of bacterial proliferation, where higher absorbance values indicate more active bacterial growth. As presented in Figure 1, among the fruit-based waste media, banana peel extract had the greatest support absorbance values, indicating that it had more conducive nutrient composition for bacterial growth. Watermelon rind had moderate support, while citrus peel had the least absorbance, showing its limited available nutrients for Bti proliferation.

3.2 Spore Observation and Viable Spore Count

According to Table 1, bacterial proliferation was highest on banana peel extract (4.39 $\log_{10}$ CFU/mL) then on watermelon rind (3.70 $\log_{10}$ CFU/mL), indicating its potential as a nutrient source due to its relatively high carbohydrate and mineral (Hikal et al., 2022; Norazman et al., 2024). In contrast, citrus peel medium presented the least viable count (2.40 $\log_{10}$ CFU/mL), implying that its limited nutrient set or presenting compounds like citric acid might have suppressed bacterial proliferation and sporulation (Hasan et al., 2022). These results are consistent with the growth curve analysis, confirming that banana peel extract provided more favorable growth conditions than the other fruit-based media tested.

Table 1 Viable Bti Spore Count in Different Food-based Waste Media

Media Type	$\log_{10}$ (CFU/mL)
TSB	7.80
Watermelon rind	3.70
Banana peel	4.39
Citrus peel	2.40

Under the Schaeffer–Fulton spore staining method, spores stained green due to the encapsulation of malachite green inside the resistant spore coat, whereas the surrounding vegetative cells were counterstained pink using safranin. This verified the facts that *Bacillus thuringiensis israelensis* not just grew but also completed sporulation in food waste-based media thus support the CFU evidence. Spore and vegetative cell presence signify complete maturity of the culture, validating cell division, as well as spore formation, on these unconventional substrates.

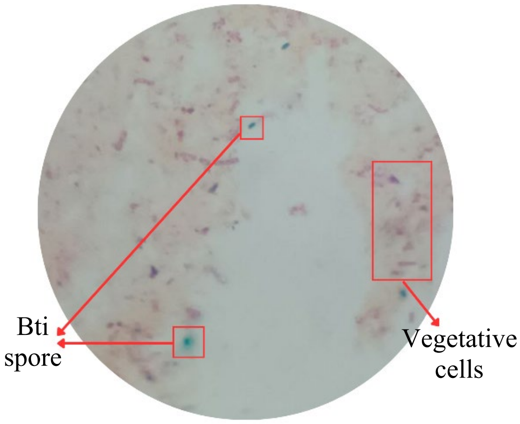

Figure 2. Visualization of *Bacillus thuringiensis israelensis* sporulation using malachite green stain.

4. CONCLUSION

Although the growth performance from fruit-based waste media maintained around half the growth vigor of the benchmark TSB, the findings prove that they can sustain viable Bti culture. The successful proliferation and sporulation of Bti on banana peel and watermelon rind extracts, respectively, identifies them as nutritionally enough for backing microbial activity. This shows their promise for being low cost, environmentally friendly substitutes for growing Bti. Further optimization of the media preparation and formulation could enhance bacterial proliferation together with spore yield, elevating the viability of commercial-scale Bti fermentation using wastewaters of food plants.

ACKNOWLEDGEMENT

The author would like to express sincere gratitude to Dr. Hazirah Pengiran and Dr. Amin Rukaini Mustafa for their invaluable guidance and supervision throughout this research. Appreciation is also extended to the Faculty of Civil Engineering & Technology and Faculty of Chemical Engineering & Technology, Universiti Malaysia Perlis (UniMAP) for providing laboratory facilities and technical assistance. This research was supported by Fundamental Research Grant Scheme (FRGS, 9003-01005) under the Ministry of Higher Education, Malaysia.

REFERENCE

[1] Hasan, Md. M., Roy, P., Alam, M., Hoque, Md. M., & Zzaman, W. (2022). Antimicrobial activity of peels and physicochemical properties of juice prepared from indigenous citrus fruits of Sylhet region, Bangladesh. Heliyon, 8(7), e09948. https://doi.org/10.1016/j.heliyon.2022.e09948.

[2] Hikal, W. M., Said-Al Ahl, H. A. H., Bratovcic, A., Tkachenko, K. G., Sharifi-Rad, J., Kačániová, M., Elhourri, M., & Atanassova, M. (2022). Banana Peels: A Waste Treasure for Human Being. Evidence-Based Complementary and Alternative Medicine, 2022, 1–9. https://doi.org/10.1155/2022/7616452.

[3] Norazman, N. A. S., Maidin, N. M., Ismail, N. A., Lani, M. N., Hamzah, Y., Salleh, F. S. M., & Isa, N. S. M. (2024). The Microbiological and Chemical Characteristics of Yoghurt Incorporated With Watermelon Rind Powder. Malaysian Applied Biology, 53(3), 95–105. https://doi.org/10.55230/mabjournal.v53i3.2804.

[4] Valtierra-de-Luis, D., Villanueva, M., Lai, L., Williams, T., & Caballero, P. (2020). Potential of Cry10Aa and Cyt2Ba, Two Minority δ-endotoxins Produced by Bacillus thuringiensis ser. israelensis, for the Control of Aedes aegypti Larvae. Toxins, 12(6), 355. https://doi.org/10.3390/toxins12060355.