

Cultivation-dependent transcriptomic reprogramming and insecticidal performance of *Beauveria bassiana* A1 from solid-state and submerged cultures

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Abstract

Background

Beauveria bassiana is an important entomopathogenic fungus widely used in microbial pest management. Solid-state fermentation (SSF) is the predominant production method, whereas submerged liquid fermentation (SLF) offers potential advantages in process control and scalability. However, how cultivation conditions affect fungal transcriptional programs and the insecticidal performance of the resulting spores remains insufficiently understood. This study compared the transcriptomic profiles and insecticidal efficacy of *B. bassiana* A1 produced by SSF and SLF against the diamondback moth, *Plutella xylostella*.

Results

Transcriptomic analysis with a false discovery rate (FDR) < 0.05 revealed pronounced transcriptional divergence between solid-state (A1-S) and submerged (A1-L) cultures. A1-S showed higher expression of genes associated with secondary metabolism and extracellular degradation, including all differentially expressed PKSs (4/4), most NRPSs (6/8), hydrolases (112/168), proteases (52/89), and chitinases (5/7). The cuticle-degrading subtilisin-like protease bassiasin I (BBA_00443) was 32.4-fold higher in A1-S (FDR = 2.10×10^{-16}). In contrast, A1-L preferentially expressed iron-acquisition and selected host-interaction-associated genes. Bioassays demonstrated concentration-dependent mortality for both spore types. At 10^7 spores/mL, A1-S showed a tendency toward faster early mortality, but the difference from A1-L was not statistically significant after Benjamini–Hochberg adjustment ($P = 0.08814$). At 10^8 spores/mL, both spore types achieved 100% mortality by day 3, with no significant difference between them ($P = 0.83504$).

Conclusion

Cultivation conditions induced substantial transcriptomic reprogramming in *B. bassiana* A1, particularly in extracellular degradation and secondary metabolism. These differences were associated with differences in the timing of host mortality rather than ultimate insecticidal efficacy. The ability of submerged conidia to achieve comparable mortality at high propagule density supports further evaluation of SLF as an alternative production platform for *B. bassiana* biopesticides, particularly when formulation and application strategies are optimized to compensate for differences in early infection dynamics.

1. Introduction

The production process is an important determinant of the biological properties of *B. bassiana* propagules. Two major production strategies are solid-state fermentation (SSF) and submerged liquid

fermentation (SLF), which provide fundamentally different physical and nutritional environments and consequently promote distinct fungal developmental states (Mascarin et al. 2024). SSF, generally performed on moist cereal grains or agricultural substrates, is widely used for aerial conidia production. The relatively low water availability and heterogeneous gas–solid environment promote aerial growth and conidiation. Aerial conidia possess specialized surface structures, including hydrophobins and rodlet layers, that contribute to hydrophobicity, adhesion, environmental persistence, and host interaction (Feng et al. 2025; Holder and Keyhani 2005). SSF is attractive because it uses relatively inexpensive substrates and simple production systems and yields propagules well suited for dry formulations. However, heterogeneous heat and mass transfer, spatial variation in moisture and oxygen, contamination risks, and difficulties in process control and scale-up remain major limitations (Mascarin et al. 2024; Wu et al. 2025).

In contrast, SLF enables more precise control of pH, dissolved oxygen, nutrient availability, agitation, and other critical process parameters, facilitating rapid biomass production, automation, and improved batch-to-batch consistency (Mascarin et al. 2024). However, submerged cultivation can produce propagules that differ substantially from aerial conidia, including blastospores and submerged conidia. These propagules exhibit distinct morphological, physiological, and surface characteristics and may differ in environmental tolerance and insecticidal activity. Iwanicki et al. (2023) demonstrated that the yield and virulence of submerged conidia, blastospores, and aerial conidia varied among *B. bassiana* isolates, emphasizing that the choice of propagule is an important consideration in microbial pesticide development.

The contrasting environments imposed by SSF and SLF are expected to induce extensive physiological and transcriptional reprogramming. During SLF, fungal cells develop under relatively uniform hydration (Iwanicki et al. 2023), rapid nutrient diffusion (Lee and Ra 2022), controlled oxygen availability, and mechanical shear, whereas SSF exposes hyphae to heterogeneous substrates (Yafetto 2022), restricted water activity (Gervais and Molin 2003), localized nutrient and oxygen gradients, and extensive gas-solid interfaces (Obi 2019). Such differences may alter fungal morphogenesis (Lu et al. 2024), stress responses (Aloo et al. 2025; Wu et al. 2021), primary and secondary metabolism (Robinson et al. 2001; Bills and Gloer 2016), cell-wall remodeling (Abe 2025), and extracellular enzyme production (Bamidele et al. 2025). Previous transcriptomic studies have shown that environmental and host-derived signals can strongly modify gene expression in *B. bassiana*, including genes associated with cuticle degradation, metabolism, and pathogenicity (Mantilla et al. 2012). Moreover, recent comparative secretomic analyses revealed substantial differences in extracellular protein profiles between SSF and SLF, with SSF producing greater numbers of carbohydrate-active enzymes and proteases (Amobonye et al. 2025). These findings suggest that fermentation conditions may influence not only propagule morphology but also the molecular and functional characteristics relevant to fungal fitness and insect pathogenicity.

Despite these advances, the molecular mechanisms linking fermentation conditions to the functional phenotype and insecticidal performance of *B. bassiana* remain insufficiently characterized. In particular, integrated comparisons of cultivation-dependent transcriptional changes involving secondary

metabolism, extracellular enzymes, host-interaction functions, and the biological activity of the resulting propagules are still limited.

Therefore, the present study investigated cultivation-dependent transcriptomic reprogramming in *Beauveria bassiana* strain A1 under solid-state (A1-S) and submerged liquid (A1-L) fermentation. Using a transcriptomic dataset comprising 10,364 genes, we characterized differentially expressed genes, with particular emphasis on secondary metabolism (PKS and NRPS), extracellular enzymes (hydrolases, proteases, and chitinases), and host-interaction and virulence-associated functions. We further compared the insecticidal performance of aerial and submerged conidia against the diamondback moth, *Plutella xylostella*. By integrating transcriptomic responses with insecticidal performance, this study provides insights into the effects of cultivation conditions on the functional characteristics of *B. bassiana* and their implications for microbial biopesticide development.

2. Material and Methods

2.1 Fungal strain and preparation of samples for transcriptomic analysis

Beauveria bassiana A1, a wild-type strain obtained from the Agricultural Chemicals Research Institute (ACRI), Taichung, Taiwan, was used in this study. The fungus was routinely maintained on V8 agar plates containing 10% V8 juice (Campbell's, USA), 2% Bacto Agar (BD Difco™, USA), and 0.2% CaCO₃ (Scharlau, Spain) at 28°C. For long-term preservation, the strain was stored as a 10% glycerol stock at -80°C at ACRI.

For the production of aerial conidia, *B. bassiana* A1 was cultured on V8 agar plates at 28°C for 7 days. Aerial conidia and associated hyphal material were subsequently collected from the plates and used for transcriptomic analysis. For submerged cultivation, an aerial conidial suspension was prepared at a concentration of 1×10^8 conidia/mL and used as the inoculum. The suspension was inoculated at 1% (v/v) into 250 mL of submerged medium according to Iwanicki et al. (2023), followed by incubation at 150 rpm in the dark. Submerged conidia were harvested after 4 days of cultivation.

For each cultivation condition, fungal samples were collected from two independent biological replicates for subsequent RNA extraction and next-generation sequencing (NGS). Thus, the transcriptomic analysis included samples representing the aerial conidia/hyphal state produced under solid-state cultivation and the submerged conidia state produced under submerged cultivation.

2.2 Illumina Sequencing for Transcriptomic Analysis

Fungal samples derived from solid-state and submerged cultures were harvested, immediately frozen in liquid nitrogen, and ground to a fine powder. Total RNA was extracted using the Quick-RNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The quantity and integrity of the purified RNA were assessed using a Qubit® 2.0

Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), respectively. For each sample, 1 µg of total RNA was used for library preparation using the Universal Plus mRNA-Seq with NuQuant platform (TECAN, Männedorf, Switzerland), following the manufacturer's protocol. The concentration and quality of the resulting libraries were evaluated using three complementary approaches. Library DNA concentration was measured using a Qubit® 2.0 Fluorometer with the QuantiFluor® dsDNA System (Promega Corporation, Madison, WI, USA). Library size distribution and integrity were assessed using an Agilent 2100 Bioanalyzer with the Agilent DNA 1000 Reagents and DNA 1000 Kit (Agilent Technologies, Santa Clara, CA, USA). Quantitative PCR (qPCR) using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) was performed to determine the effective adapter-containing library concentration. Libraries that met the required quality criteria were subsequently subjected to high-throughput sequencing. Paired-end sequencing (2 × 151 bp) was performed on an Illumina NovaSeq X Plus Sequencing System (Illumina, San Diego, CA, USA) at Tri-I Biotech Inc. (Taipei, Taiwan). Low-quality reads were removed using the Illumina data-processing pipeline. Raw read counts were used as input for differential expression analysis using DESeq2. For descriptive visualization of gene expression levels, FPKM values were calculated using CLC Genomics Workbench (QIAGEN Digital Insights, Aarhus, Denmark). Differentially expressed genes (DEGs) between solid-state- and submerged-culture-derived samples were initially identified based on an absolute log₂ fold change ($|\log_2FC|$) > 1 and a raw P-value < 0.05 using DESeq2 (R package). To account for multiple testing, the raw P-values obtained from the differential expression analysis were subsequently adjusted using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995), and the resulting false discovery rate (FDR)-adjusted P-values were used for statistical significance assessment. Genes with $|\log_2FC|$ > 1 and FDR < 0.05 were considered significantly differentially expressed.

2.3 Preparation of Conidial Suspensions for insecticidal effects

The aerial conidia of *Beauveria bassiana* A1 were produced on V8 agar plates and incubated for 7 days. The conidia were subsequently harvested and suspended in 0.1% Tween 80 solution. Submerged conidia were produced by culturing *B. bassiana* A1 in submerged medium for 4 days, after which the culture broth containing the conidia was collected. Conidial concentrations were determined and adjusted using a Neubauer hemocytometer (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Conidial viability was determined prior to the bioassays, and only conidial preparations with a viability exceeding 90% were used in subsequent experiments.

2.4 Bioassay against diamondback moth

Seven treatments were included in the bioassay against the diamondback moth, *Plutella xylostella* (Linnaeus), consisting of a control and three concentrations (10⁶, 10⁷, and 10⁸ conidia/mL) of aerial and submerged conidia of *Beauveria bassiana* A1 strain. Diamondback moth larvae were obtained from the insectary of the Agricultural Chemicals Research Institute (ACRI), Taiwan.

For each treatment, 1 mL of the conidial suspension was applied to a fresh cabbage leaf disc (5 cm in diameter) using a Potter spray tower (Burkard Manufacturing Co. Ltd., UK) at a pressure of 4 lb/in². The treated leaf discs were then allowed to air-dry. After the leaf surface had completely dried, six larvae were transferred onto each leaf disc, and one leaf disc was placed in each Petri dish. For the control treatment, the leaf discs were sprayed with 0.1% Tween 80 solution.

The treated larvae were maintained at $28 \pm 1^\circ\text{C}$ under a 12:12 h light:dark photoperiod for 5 days. Larval mortality was recorded daily, and dead larvae were collected and surface-sterilized with 70% ethanol. The cadavers were subsequently placed in a humid chamber to induce fungal sporulation, and mycosis was confirmed based on the development of characteristic fungal growth and sporulation. Each treatment consisted of four replicates, with six larvae per replicate. The entire bioassay was independently repeated four times.

2.5 Median lethal time (LT₅₀) determination and statistical analysis

Each treatment consisted of four replicate Petri dishes, with six larvae per dish ($n = 24$ larvae per treatment). The experiment was conducted independently four times. Cumulative larval mortality was recorded daily. Median lethal time (LT₅₀) values and their associated 95% confidence intervals (95% CIs) were calculated by probit analysis using generalized linear models (GLMs, binomial error distribution with a probit link function). The 95% CIs were determined according to Fieller's method. The goodness-of-fit for each regression line was evaluated using chi-square (χ^2) tests. Survival dynamics among treatments were compared using Kaplan-Meier survival curves and pairwise log-rank tests with Benjamini-Hochberg adjustment.

All statistical analyses were conducted using R software (version 4.3.3, R Foundation for Statistical Computing, Vienna, Austria) with the survival /survminer and drc /MASS package.

3. Results

3.1 Transcriptomic reprogramming in response to cultivation mode

The cultivation mode induced extensive transcriptional reprogramming in *Beauveria bassiana* A1. As shown in Table 1, comparison of the transcriptomes obtained from solid-state (A1-S) and submerged (A1-L) cultivation identified 4,442 genes showing significant differences in expression ($\text{FDR} < 0.05$) among the 10,364 genes analyzed. Of these, 2,203 genes exhibited higher expression under solid-state cultivation, whereas 2,239 genes showed higher expression under submerged cultivation. The broadly distributed transcriptional changes indicate that the cultivation environment was associated with substantial changes in the transcriptional state of *B. bassiana* A1 rather than affecting a single metabolic pathway. In particular, differential expression was observed among genes associated with secondary

metabolism, extracellular hydrolytic activity, proteolysis, chitin degradation, and host-associated functions, suggesting that cultivation mode may influence both fungal metabolism and traits potentially relevant to subsequent host infection.

3.2 Differential expression of hydrolytic enzyme genes under solid-state and submerged cultivation

Solid-state culture (A1-S, producing aerial/hyphal state) and submerged liquid culture (A1-L) displayed widespread transcriptomic remodeling (FDR < 0.05). As shown in Fig. 1, among 356 annotated hydrolase genes, 168 were differentially expressed genes (DEGs), with 112 upregulated in A1-S versus 56 in A1-L. Similar directional biases were observed for proteases (52 of 89 DEGs upregulated in A1-S) and chitinases (5 of 7 DEGs upregulated in A1-S).

3.3 Secondary metabolic gene clusters profiling of solid-state and submerged cultures

Polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) genes showed higher transcript abundance under solid-state conditions. The molecules synthesized by PKS and NRPS are generally not essential for primary cellular maintenance (Gerald and James 2016), yet they play a crucial role in ecological competition, defense, parasitism, and symbiosis. Refer to supplementary Table 1 and Fig. 1, all four PKS DEGs were markedly upregulated in solid-state culture (A1-S), exhibiting up to 71.3-fold (BBA_03616), 38.9-fold (BBA_01841), and 20.7-fold (BBA_05176, hybrid NRPS/PKS) higher expression compared to submerged culture (A1-L).

3.4 Divergent host-interaction and virulence determinants profiling of solid-state and submerged cultures

As shown in supplementary Table 1 and Fig. 1, for A1-S-biased expression, genes encoding proteins potentially involved in cuticular interaction and penetration were preferentially transcribed in A1-S, most notably the cuticle-degrading protease bassiasin I precursor (BBA_00443, 32.4-fold higher in A1-S) and the MFS toxin efflux pump AflT (BBA_09719, 28.7-fold higher in A1-S).

In contrast, for A1-L-biased expression, submerged culture promoted genes associated with iron acquisition and toxin-associated functions, including low-affinity and siderophore iron transporters (BBA_00449 and BBA_09226, the latter upregulated 43.2-fold), the siderophore synthetase SidC (BBA_00963), and specific toxin homologs (e.g., structural toxin RtxA BBA_03189 and mosquitocidal toxin BBA_01257).

3.5 Insecticidal effects of aerial conidia and submerged conidia on diamond-back moth larvae

Both aerial spores and submerged spores of *B. bassiana* A1 exhibited marked dose-dependent insecticidal activity against diamondback moth (DBM, *Plutella xylostella*) larvae. As shown in Fig. 2, at

the highest inoculation concentration (10^8 spores/mL), both spore types induced substantial larval mortality by days 2 to 3, differing significantly from the Tween 80 control (pairwise log-rank test, aerial spores $p = 0.000037$; submerged spores $p = 0.000027$) (supplementary Table 1).

In Fig. 3, survival analysis revealed significant differences in the mortality kinetics of *Plutella xylostella* across different spore types and concentrations (Log-rank test, $p < 0.0001$). High-concentration treatments (10^8 spores/mL) of both aerial and submerged spores demonstrated the most rapid insecticidal activity, with survival rates of approximately 60% by day 2 (60.3% and 58.0%, respectively) and reaching about 20% by day 3. At moderate and lower concentrations (10^7 and 10^6 spores/mL), all fungal treatments crossed the median lethal threshold (LT_{50}) on day 3. Aerial spores at 10^7 spores/mL elicited faster initial knockdown by day 2 (survival rate 70.5%), whereas submerged spores exhibited a delayed surge, with survival sharply dropping between days 2 and 3. In contrast, the control group maintained over 69% survival until day 3 and only crossed the LT_{50} threshold at day 4.

3.6 Median lethal time (LT_{50}) of aerial conidia and submerged conidia

The median lethal time (LT_{50}) of *Beauveria bassiana* A1 against *Plutella xylostella* decreased markedly in a dose-dependent manner. As shown in Fig. 4, at the highest concentration (10^8 spores/mL), killing kinetics between the two spore morphotypes converged, yielding nearly identical LT_{50} values of 1.41 days (aerial conidia) and 1.32 days (submerged spores). However, at the intermediate concentration (10^7 spores/mL), a distinct divergence was observed. Aerial conidia achieved an LT_{50} of 1.69 days, whereas submerged spores required 2.24 days, corresponding to a 0.55-day shorter LT_{50} for aerial conidia. At 10^6 spores/mL, speed of kill was protracted in both preparations ($LT_{50} = 2.50$ and 2.24 days, respectively).

4. Discussion

Beauveria bassiana is a prominent and widely used entomopathogenic fungus for the biological control of a broad range of insect pests (Jaronski and Mascarín 2017). Although solid-state fermentation (SSF) remains the predominant approach for commercial production, biphasic and submerged liquid fermentation (SLF) technologies have progressively matured and may provide greater opportunities for process intensification and scale-up (Li et al. 2022; Pham et al. 2010). Because cultivation conditions can influence both fungal physiology and downstream production processes (Jaronski and Mascarín 2017), the present study compared the transcriptomic profiles and insecticidal performance of *B. bassiana* A1 produced under solid-state and submerged cultivation.

Based on the $FDR < 0.05$ reanalysis, *B. bassiana* A1 exhibited a pronounced transcriptional divergence between solid-state (A1-S) and submerged (A1-L) cultivation. Solid-state cultivation genes showed

higher expression associated with secondary metabolism and extracellular degradation, including all differentially expressed polyketide synthases (PKSs, 4/4), most nonribosomal peptide synthetases (NRPSs, 6/8), hydrolases (112/168), proteases (52/89), and chitinases (5/7). In contrast, submerged cultivation showed higher expression of genes associated with iron acquisition, secretory processing peptidases, and selected toxin-associated functions. Among the 19 significant host-interaction-associated genes, 13 were related to iron acquisition, including SidC, MirB, and multiple iron permeases.

These findings indicate that the two cultivation systems impose distinct physiological environments that promote different transcriptional programs. The liquid-culture response is also consistent with previous observations that osmotic and hydrodynamic stresses associated with submerged cultivation can activate cytosolic and endoplasmic reticulum chaperone systems, including Ssb1, Ssz1, and STI1, to maintain protein homeostasis and support morphological adaptation (Mascarin et al. 2024). Similarly, Wei et al. (2024) demonstrated that environmental sensing, morphological differentiation, and secondary metabolic pathways can undergo substantial transcriptional reprogramming between different cultivation environments, despite relative conservation of core cellular functions.

The transcriptional divergence observed between A1-S and A1-L may have important implications for the early stages of host infection. Aerial conidia produced under solid-state conditions possess a hydrophobic surface associated with a rodlet layer formed by class I hydrophobins, which facilitates adhesion to the waxy epicuticle of insect hosts (Ortiz-Urquiza and Keyhani 2013; Zhang et al. 2011). In addition to these surface properties, the elevated transcription of extracellular hydrolytic enzymes observed in A1-S suggests that solid-state cultivation may place the fungus in a transcriptionally prepared state for subsequent degradation of host cuticular barriers.

In particular, the cuticle-degrading subtilisin-like protease bassiasin I (BBA_00443) was strongly upregulated in A1-S, showing a 32.4-fold increase with an FDR of 2.10×10^{-16} . Class III chitinase ChiA2 (BBA_05353) was also differentially expressed (FDR = 8.09×10^{-8}), while 5 of the 7 differentially expressed chitinases, all 4 differentially expressed PKSs, and 6 of 8 NRPSs were preferentially expressed under solid-state cultivation. Collectively, these transcriptional patterns suggest a transcriptional bias toward extracellular degradation and secondary metabolism under solid-state cultivation.

Submerged propagules, in contrast, develop under a physiologically distinct liquid environment and exhibit different surface characteristics from aerial conidia (Holder et al. 2007; Jeffs et al. 2011; Mascarin and Jaronski 2016). The lower expression of many extracellular hydrolytic genes in A1-L therefore suggests that submerged cultivation is associated with lower transcriptional representation of extracellular hydrolytic functions for immediate extracellular degradation. However, this difference should not be interpreted as evidence that submerged propagules are intrinsically incapable of efficiently infecting insects. Rather, it may primarily influence the timing and relative contribution of different infection processes.

The bioassay results provide an important functional context for interpreting these transcriptomic differences. Both aerial and submerged spores produced a clear concentration-dependent insecticidal

response against *P. xylostella*. According to the Log-rank analysis with Benjamini–Hochberg adjustment, both 10^8 aerial spores/mL and 10^8 submerged spores/mL significantly accelerated larval mortality relative to the Tween 80 control ($P = 3.7 \times 10^{-5}$ and 2.7×10^{-5} , respectively). Importantly, the survival curves of the two spore types at 10^8 spores/mL did not differ significantly ($P = 0.83504$). Both treatments reached 100% cumulative mortality by day 3.

A similar pattern was observed at 10^7 spores/mL, although the difference between aerial and submerged spores was not statistically significant. The pairwise Log-rank comparison gave $P = 0.08814$ after BH adjustment. Nevertheless, the raw mortality data indicate a tendency toward faster early mortality in the aerial-spore treatment. By day 2, 15 of 24 larvae had died in the 10^7 aerial-spore treatment, compared with 7 of 24 in the 10^7 submerged-spore treatment. By day 3, mortality had increased to 23/24 (95.8%) and 22/24 (91.7%), respectively. Thus, the data suggest a possible difference in the timing of mortality, rather than a definitive difference in ultimate insecticidal efficacy.

Transcriptomic differences should therefore be interpreted as indicators of physiological and infection-related preparedness rather than as direct evidence of virulence. Previous transcriptomic studies of *B. bassiana* have shown that differences in the expression of cuticle-degrading enzymes, cytochrome P450s, adhesion-related proteins, and other pathogenicity-associated genes can be associated with contrasting virulence phenotypes and may reflect differences in the timing or route of pathogenicity-related metabolism (Sessa et al. 2024). The elevated expression of cuticle-degrading proteases and chitinases in A1-S provides a plausible molecular basis for may be consistent with more rapid initiation of host invasion, whereas the alternative functional profile of A1-L, characterized by stronger representation of iron-acquisition and selected toxin-associated genes, may support subsequent infection processes. However, these mechanisms remain hypotheses unless supported by direct measurements of enzyme activity, siderophore production, toxin abundance, or fungal development within the host.

The convergence of insecticidal efficacy at 10^8 spores/mL is particularly informative. Despite substantial transcriptomic differences between A1-S and A1-L, both spore types caused complete mortality by day 3, and their survival curves were statistically indistinguishable ($P = 0.83504$). This result suggests that transcriptional differences in extracellular degradation and secondary metabolism do not necessarily translate into proportional differences in final insect mortality under high-inoculum conditions.

One possible explanation is that a sufficiently high propagule density increases the probability of successful host attachment and penetration, thereby reducing the functional consequences of differences in the efficiency of individual propagules. Under these conditions, even if submerged spores have a relatively lower transcriptional investment in cuticle-degrading enzymes before host contact, the high number of propagules applied to the host may compensate for reduced efficiency at the individual-spore level.

The findings at 10^7 spores/mL further support this interpretation. Although aerial spores produced a noticeably faster mortality response during the early infection period, the overall difference remained

statistically nonsignificant ($P = 0.08814$), and mortality converged to approximately 92 to 96% by day 3. Thus, the one potential consequence of the cultivation-dependent transcriptional divergence may be variation in the timing of host mortality.

This distinction has practical significance for microbial pesticide development. Aerial conidia may have a potential advantage when rapid mortality is required when rapid infection is required or when application rates are constrained, whereas submerged conidia may achieve comparable final efficacy when sufficient propagule density can be delivered. This provides a biological rationale for considering SLF-derived propagules as an alternative production platform rather than assuming that their different transcriptomic state necessarily results in inferior biocontrol performance.

From a production perspective, these results highlight an important trade-off between cultivation-dependent fungal physiology and application performance. SSF remains highly suitable for producing aerial conidia with physiological characteristics favorable for rapid interaction with insect cuticles. However, SLF offers potential advantages associated with process control, shorter cultivation cycles, reduced manual handling, and scalability (Mascarin and Jaronski 2016; Pham et al., 2010). The present results indicate that the distinct transcriptional state associated with SLF did not result in a detectable reduction in insecticidal activity under the conditions tested, particularly when sufficient propagule density is applied.

Nevertheless, the potential economic advantage of SLF should not be interpreted as a demonstrated reduction in total production costs because a complete economic analysis was not performed in the present study. Rather, the results provide evidence that production efficiency and biological efficacy may be optimized independently: the cultivation process can be selected according to manufacturing requirements, while formulation and application strategies can be optimized to compensate for differences in early infection kinetics.

Future studies should therefore evaluate not only mortality but also conidial adhesion, germination, cuticle penetration, enzyme activity, persistence on plant surfaces, formulation stability, and field efficacy. Such integrated analyses would help determine whether the higher scalability of SLF can be translated into a practical advantage for commercial *B. bassiana* biopesticides.

5. Conclusion

Solid-state and submerged cultivation induced pronounced transcriptomic divergence in *B. bassiana* A1, with solid-state cultivation favoring secondary metabolism and extracellular hydrolytic functions, whereas submerged cultivation preferentially expressed iron-acquisition and selected host-interaction-related functions. These molecular differences were associated with differences in the timing of insect mortality, but not necessarily with final insecticidal efficacy. At 10^7 spores/mL, aerial conidia showed a faster early mortality tendency than submerged conidia, although the difference was not statistically significant after BH adjustment ($P = 0.08814$). At 10^8 spores/mL, both spore types achieved 100% mortality by day 3, with no significant difference between them ($P = 0.83504$).

Overall, the results suggest that cultivation-dependent transcriptomic reprogramming is associated with differences in the timing of host mortality rather than with a detectable difference in ultimate insecticidal efficacy under the conditions tested. These findings support further evaluation of submerged fermentation as an alternative production strategy, particularly when high propagule density, formulation optimization, and appropriate application strategies can be used to compensate for differences in early infection dynamics.

Declarations

Author Contribution

YR Liang and JH Yen planned the study; YR Liang conducted the laboratory experiments and wrote the manuscript; JH Yen reviewed the manuscript.

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Tables

Table 1
Differentially expressed genes in *Beauveria bassiana* A1 strain (FDR < 0.05, |FC| ≥ 2)

Items	Upregulated in submerged fermentation	No significant change	Upregulated in solid-state fermentation
Gene numbers	2,239	5,922	2,203
Percentage of DEGs* (%)	50.41%	-	49.59%
Functions	Osmotic, oxidative, heat shock proteins (HSP)	Core genes, housekeeping genes	Conidiation, CAZymes
*DEGs: Differentially Expressed Genes			

Figures

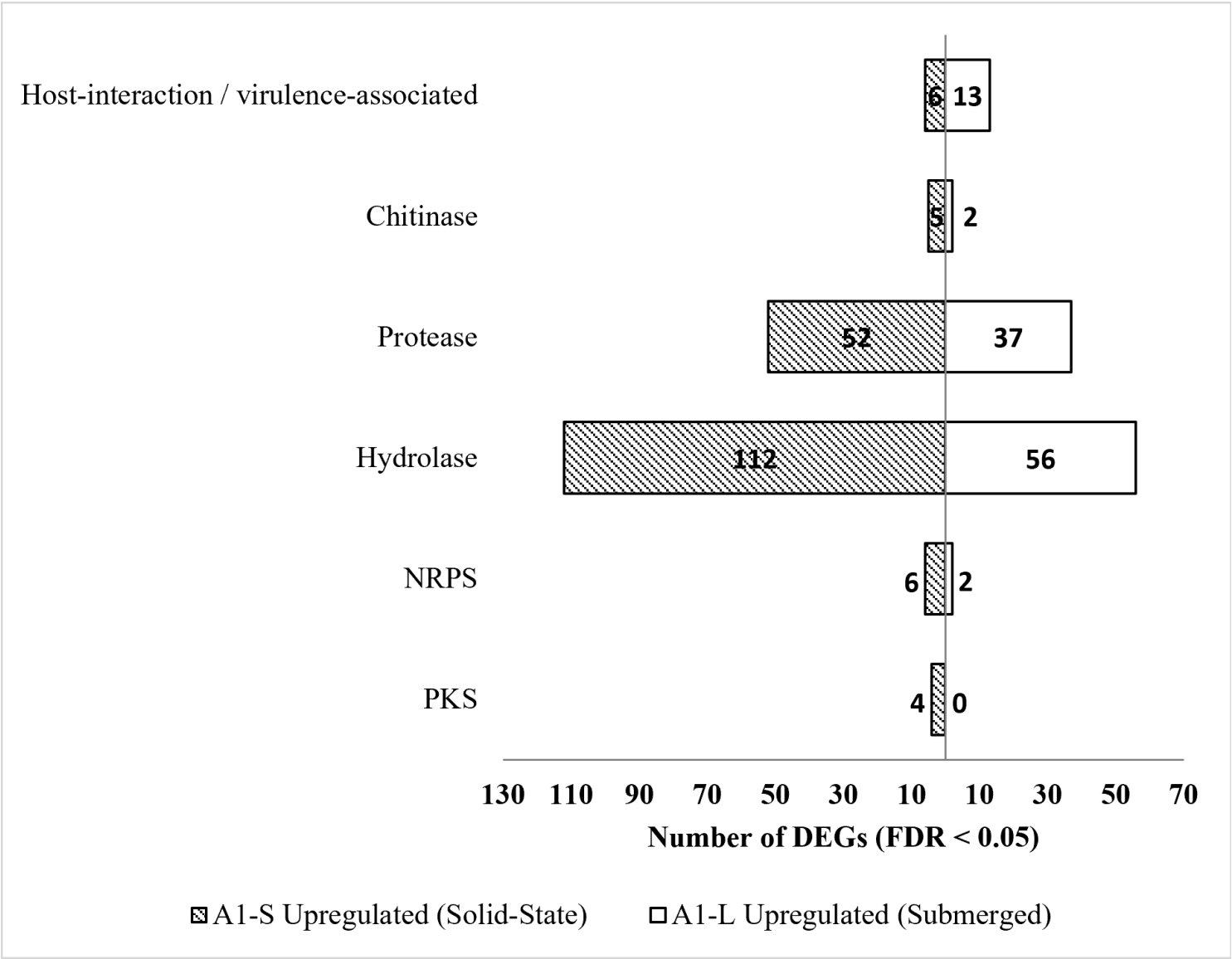


Figure 1

Functional category distribution of differentially expressed genes (DEGs) in *Beauveria bassiana* A1 between solid-state and submerged cultivations

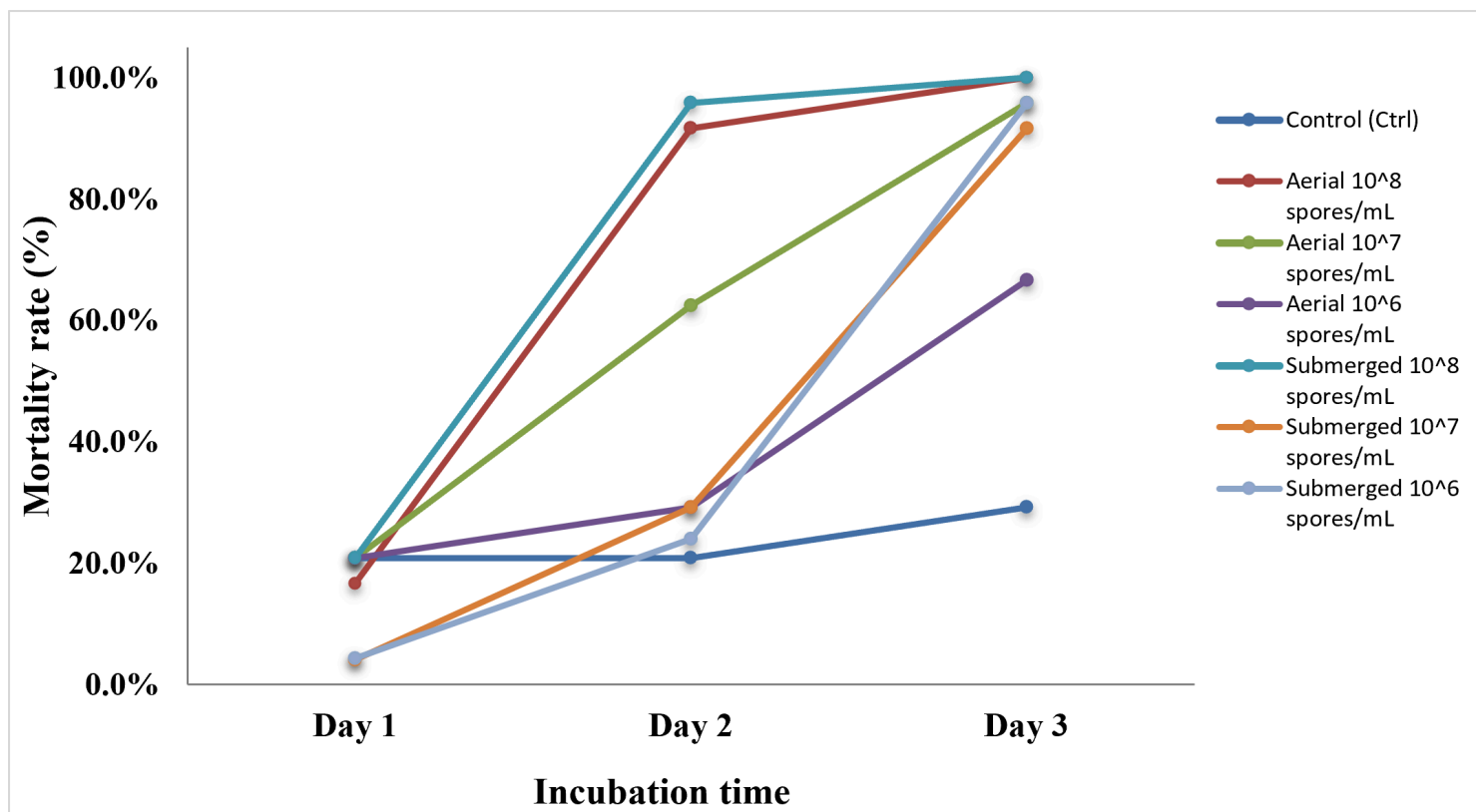


Figure 2

Comparison of cumulative mortality rates of *Plutella xylostella* between aerial and submerged spores at different concentrations over time

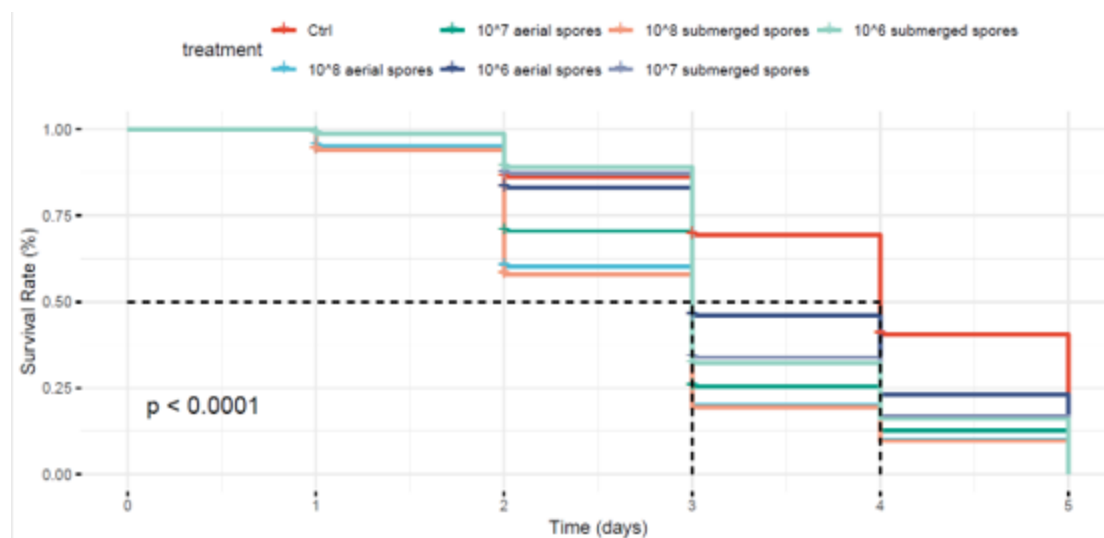


Figure 3

Kaplan-Meier survival curves of *Plutella xylostella* treated with aerial and submerged spores of *Beauveria bassiana*A1 at different concentrations

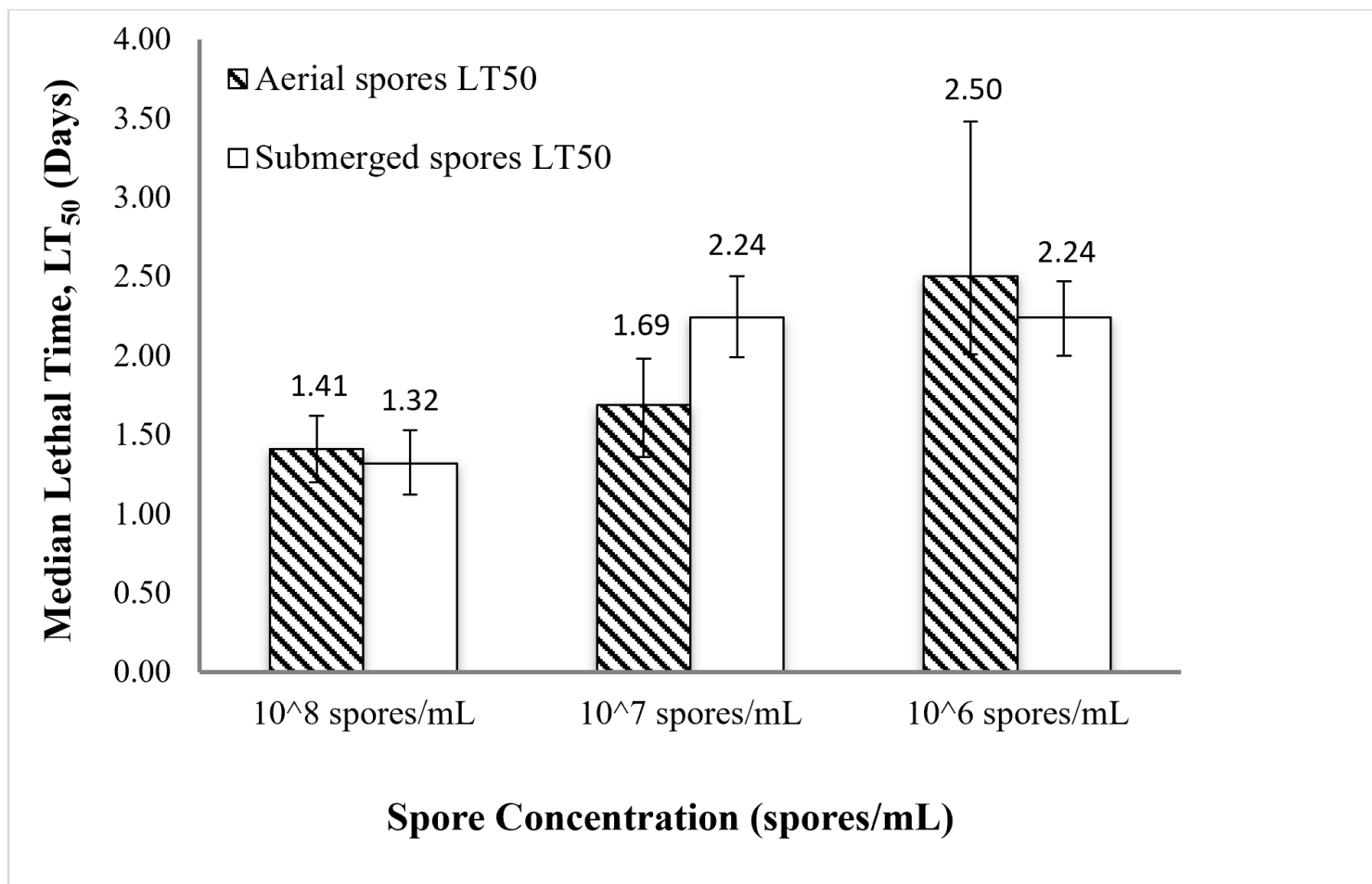


Figure 4

Median lethal time (LT₅₀) by spore type and concentration. Error bars represent the 95% confidence intervals of the LT₅₀ estimates. Asterisks indicate statistically significant differences (p < 0.01).

Supplementary Files

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- [SupplementaryData1FunctionalDEGsF.xlsx](#)
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