fermentation

MDPI

Article

Amino Acid Elicitation for Improved Hypocrellin A Biosynthesis in *Shiraia bambusicola* Under Elevated Fermentation Temperature

Qiulin Wei [1], Yongdi Wen [1], Jialu Liu [1], Fuqiang Yu [2], Zhenqiang Wu [1] and Xiaofei Tian [1,3,*]

[1] Guangdong Engineering Research Center of Low-Carbon Synthetic Biotechnology, School of Biology and Biological Engineering, South China University of Technology, University Park, Guangzhou 510006, China; 202420148540@mail.scut.edu.cn (Q.W.); 202020148989@mail.scut.edu.cn (Y.W.); 202520148294@mail.scut.edu.cn (J.L.); btzhqwu@scut.edu.cn (Z.W.)

[2] Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China; fqyu@mail.kib.ac.cn

[3] Zhuhai Institute of Modern Industrial Innovation, South China University of Technology, Zhuhai 519100, China

* Correspondence: xtien@scut.edu.cn

Abstract

Hypocrellin A (HA) is a perylenequinone photosensitizer produced by *Shiraia bambusicola* during submerged fermentation, but its accumulation during submerged fermentation remains limited by low biosynthetic efficiency. Building on our previous finding that 32 °C increased HA accumulation and induced broad transcriptional changes, we reanalyzed that transcriptome to guide amino acid (AA) supplementation. Five AAs (Ala, Lys, Gly, Val, and Ile) were selected from eight residues showing nominal enrichment in proteins encoded by 32 °C upregulated genes because their catabolic routes are among those connected to central carbon metabolism and the generation of acetyl-CoA, the immediate precursor of the HA polyketide backbone. Single AAs and binary combinations were screened for their effects on intracellular HA content in *S. bambusicola* GDMCC 60438. At 32 °C, single supplementation with Ala (50 mg/L), Lys (200 mg/L), or Gly (100 mg/L) increased intracellular HA content by 71%, 74%, and 36%, respectively. Three binary combinations, Ala + Lys, Ile + Gly, and Val + Gly, further enhanced intracellular HA content by 71%, 75%, and 80%, respectively, with Val + Gly producing the largest increase in intracellular HA content despite Val alone being inactive. The AA response was markedly weaker at 28 °C, with larger response magnitudes observed at 32 °C in separate experiments. Transcriptomic re-analysis of previously published data revealed upregulation of AA transporter genes, including amino acid permeases (AAPs) and major facilitator superfamily (MFS) transporters, at 32 °C. Proteins encoded by highly expressed genes at 32 °C showed enrichment in eight AAs at an unadjusted $p < 0.05$, seven of which remained significant after Benjamini–Hochberg correction ($q < 0.05$). Intracellular acetyl-CoA was elevated in AA-supplemented cultures during the early fermentation phase (0–12 h). These findings support a working model in which elevated temperature is associated with a metabolically permissive state under which targeted AA supplementation may enhance HA biosynthesis in independent experiments.

Keywords: *Shiraia bambusicola*; hypocrellin A; exogenous amino acids; acetyl-CoA; polyketide biosynthesis; transcriptomic analysis

Check for updates

Academic Editor: Seraphim Papanikolaou

Received: 15 July 2026
Revised: 4 October 2026
Accepted: 8 October 2026
Published: 12 October 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Hypocrellin A (HA) is a representative perylenequinone photosensitizer produced mainly by the medicinal fungus *Shiraia bambusicola*. Owing to its excellent photodynamic activity, HA has attracted considerable attention for applications in photodynamic therapy, antimicrobial treatment, and functional photosensitizers [1,2]. Compared with extraction from natural stromata or chemical synthesis, submerged fermentation provides a more controllable and scalable approach for HA production [3–5]. However, the industrial application of HA remains constrained by its relatively low accumulation during fermentation. Consequently, the development of effective fermentation regulation strategies to improve HA biosynthesis has become an important research objective. Independent studies have reported that L-valine supplementation (1.5 g/L) increased HA to 237.92 mg/L (2.12-fold vs. the control) and that a purified bamboo polysaccharide elicitor (10 mg/L, added on day 3) increased HA to 422.8 mg/L (approximately 4.0-fold vs. the control) in other *Shiraia* culture systems [6,7]. Co-culture, urea supplementation, and microparticle-assisted cultivation have also improved intracellular HA content in *S. bambusicola* GDMCC 60438 [8–10]. These findings indicate that HA biosynthesis is responsive to environmental and nutritional regulation, while the magnitude and mechanism of the response are strongly condition-dependent.

Temperature is one of the most influential environmental factors governing fungal physiology and secondary metabolism. In a previous study by our group, cultivation of *S. bambusicola* GDMCC 60438 at 32 °C significantly increased intracellular HA content compared with conventional fermentation temperatures of 26–28 °C and was accompanied by marked alterations in mycelial morphology, global transcriptional profiles, and the expression of HA biosynthetic and transporter-related genes [4]. Temperature-dependent regulation of secondary metabolism has also been documented in other filamentous fungi, indicating that elevated temperature triggers extensive metabolic reprogramming rather than simply enhancing the expression of individual biosynthetic genes [11]. Nevertheless, the metabolic basis by which high-temperature fermentation promotes HA biosynthesis has remained largely unresolved.

Re-examination of the previously published transcriptomic dataset [4] showed that genes involved in amino acid (AA) transport and AA metabolism were among the most significantly upregulated functional categories under high-temperature fermentation. In particular, multiple AAPs and MFS members exhibited markedly increased expression, implying an enhanced cellular capacity for AA uptake. In addition, proteins encoded by genes highly expressed at 32 °C displayed an altered AA composition relative to the whole proteome. These observations suggest that particular AAs might be associated with the 32 °C transcriptional program, and high-temperature fermentation may increase the physiological demand for specific AAs. These transcriptomic observations led to the hypothesis that elevated temperature creates a metabolically permissive state in which targeted AA supplementation becomes an effective strategy for enhancing HA biosynthesis in *S. bambusicola*.

Beyond their role as protein building blocks, AAs act as metabolic regulators coordinating nitrogen metabolism, central carbon metabolism, and secondary metabolite biosynthesis [12,13]. Since HA is synthesized through a polyketide pathway initiated by acetyl-CoA and malonyl-CoA [14,15], and AA catabolism feeds directly into the acetyl-CoA pool [16], AA availability may influence HA biosynthesis by multiple routes, including precursor availability, protein synthesis, and broader metabolic regulation. However, whether AA supplementation can specifically enhance intracellular HA content under the high-temperature physiological state and which AAs are most responsive remain unclear.

In view of these considerations, the present study was designed to test the hypothesis that transcriptome-predicted AA demand can be exploited as a fermentation regulation strategy for enhancing HA biosynthesis. Transcriptomic data obtained from high-temperature fermentation were reanalyzed to identify AA transporter genes and to characterize AA enrichment in proteins encoded by highly expressed genes, from which candidate AAs were selected. The effects of individual AAs and binary AA combinations on intracellular HA content were then systematically evaluated at 28 °C and 32 °C. Intracellular acetyl-CoA dynamics were also monitored to probe a possible metabolic link between AA utilization and enhanced HA biosynthesis.

2. Materials and Methods

2.1. Strain

S. bambusicola GDMCC 60438 was previously isolated in the authors' laboratory and deposited at the Guangdong Microbial Culture Collection Center (GDMCC, Guangzhou, China). The strain was maintained on potato dextrose agar (PDA) at 4 °C.

2.2. Seed Culture Preparation and Submerged Fermentation

The strain was cultivated on PDA (Guangdong Huankai Biotechnology Co., Ltd., Guangzhou, China) plates for 10 days. A mycelial-spore suspension was prepared by rinsing the plate surface with sterile water containing 0.1% (*v*/*v*) Tween-80. A 2 mL aliquot of the suspension was inoculated into a 250 mL Erlenmeyer flask containing 50 mL of potato dextrose broth (PDB, Guangdong Huankai Biotechnology Co., Ltd., Guangzhou, China). The culture was first incubated statically at 28 °C for 4–6 h, followed by shaking cultivation at 28 °C and 150 rpm for 48 h to prepare the seed culture. The seed culture was then inoculated at 10% (*v*/*v*) into a fermentation medium consisting of 4 g/L potato extract powder, 12 g/L beef extract, and 10 g/L glycerol (initial pH 6.2 ± 0.1). Submerged fermentation was carried out in 250 mL baffled Erlenmeyer flasks containing 50 mL of the fermentation medium at 26 °C, 28 °C, or 32 °C, and 150 rpm, for 72 h.

2.3. Exogenous AA Supplementation

At the onset of fermentation at 32 °C, alanine (Ala, BR, 99%), lysine (Lys, BR, 99%), glycine (Gly, AR, 99%), valine (Val, BR, 99%), and isoleucine (Ile, BR, 99%), all obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China), were individually supplemented into the fermentation medium. The same broad concentration series was applied to all five candidates to capture the stimulatory, plateau, and inhibitory phases of the dose–response within a comparable screening experiment at final concentrations of 0, 50, 100, 150, 200, 400, 800, and 1000 mg/L. These five candidate AAs were selected on the basis of the transcriptomic reanalysis described in Sections 2.5 and 2.6. The three aromatic AAs (Phe, Trp, and Tyr) were not prioritized in this initial screening, since their catabolic routes are less directly coupled to acetyl-CoA generation. According to the results of single-AA screening, effective AAs were subsequently combined in pairs and supplemented into the fermentation medium. All ten binary combinations among the five AAs were tested using Ala at 50 mg/L, Lys at 200 mg/L, Gly at 100 mg/L, Val at 400 mg/L, and Ile at 400 mg/L. The Ala, Lys, and Gly concentrations corresponded to the maxima observed in the single-AA screen, whereas 400 mg/L was selected for Val and Ile because both showed an upward, although non-significant, trend in intracellular HA content at this concentration. The binary-treatment control contained no added AA (basal medium). In addition, AAs that significantly improved intracellular HA content at 32 °C were also evaluated at 28 °C to compare the AA response between the two temperatures.

2.4. Determination of Mycelial Biomass and Intracellular HA Content

After fermentation, mycelia were harvested by vacuum filtration, washed three times with distilled water, and freeze-dried to a constant weight for biomass determination. For HA extraction, 0.05 g of freeze-dried mycelia was thoroughly ground with quartz sand and then mixed with 5 mL of dichloromethane (Damao Chemical Reagent Factory, AR, Tianjin, China), followed by ultrasonic extraction (40 kHz, 600 W) for 30 min. The extract was filtered through a 0.22 μm organic-phase membrane (Jinteng Experimental Equipment Co., Ltd., Tianjin, China) prior to HPLC analysis.

HA content was quantified using a Waters 2695 HPLC system equipped with a 2998 Photodiode Array (PDA) Detector (Waters Corporation, Milford, MA, USA) and an Agilent ZORBAX SB-C18 column (4.6 × 250 mm, 5 μm, Agilent Technologies, Inc., Santa Clara, CA, USA) maintained at 30 °C. The mobile phase consisted of methanol (Tianjin Biaoshiqi Technology Development Co., Ltd., HPLC, Tianjin, China) and 20% (*v*/*v*) aqueous acetic acid (Damao Chemical Reagent Factory, HPLC, Tianjin, China) (9:1, *v*/*v*), delivered at a flow rate of 1 mL/min. The injection volume was 10 μL, and the detection wavelength was 467 nm. HA standard (purity 99.5%) was prepared in the authors' laboratory. The calibration curve was established using HA standard concentrations of 0.5, 1.0, 2.0, 5.0, and 10.0 mg/mL. The calibration equation was $y = 2 \times 10^7 x - 404{,}358$ ($R^2 = 0.9992$), where x is the HA concentration in the extract (mg/mL), and y is the HPLC peak area. Extraction recovery was ≥95%, as determined by spike-and-recovery [4]. The intracellular HA content in mycelia was calculated according to Equation (1).

$$\text{HA content (mg/g dry mycelium)} = \frac{\text{concentration of HA (mg/mL)} \times \text{volume of the extract (mL)}}{\text{mass of the mycelial sample(g)}} \quad (1)$$

2.5. Analysis of AA Transporter Genes Highly Expressed at 32 °C

AA transport across the cell membrane is a critical step in AA metabolism and biosynthesis [17]. Transcriptomic data of *S. bambusicola* GDMCC 60438 obtained under different fermentation temperatures from a previous study [4] were reanalyzed. Differentially expressed genes among the three temperatures were identified as described previously [4]; hierarchical clustering of the 1326 DEGs distinguished 469 genes highly expressed at 32 °C as a specific cluster (Cluster 1) [4]. Genes significantly upregulated at 32 °C were annotated using the Pfam database (v35.0, https://www.ebi.ac.uk/interpro/entry/pfam/; accessed on 4 October 2026) with an E-value cutoff of $\leq 10^{-5}$ to identify genes encoding AA transporters.

2.6. Analysis of AA Composition of Proteins Encoded by Highly Expressed Genes at 32 °C

The highly expressed genes at 32 °C refer to genes identified as differentially expressed and specifically upregulated at 32 °C relative to both 26 °C and 28 °C in the previously published transcriptomic dataset [4]. Their encoded proteins form a subset of the whole-genome predicted protein set because the query lies within the background. The overlap shifts the background mean toward the query mean and therefore attenuates rather than inflates any apparent difference. For each protein, the relative frequency of each of the 20 standard AAs was calculated as the number of residues of that AA divided by the total protein length. Individual protein sequences were treated as the statistical units. For each AA, per-protein frequencies in the 32 °C upregulated set were compared with those in the predicted proteome using an independent-samples *t*-test in IBM SPSS Statistics 26. An AA was classified as enriched or depleted when its mean relative frequency was higher or lower, respectively, at a nominal two-sided $p < 0.05$, and as not significant otherwise. As these frequencies are compositional (summing to unity within each protein) and thus not mutually independent, false-discovery-rate control was applied using both the Benjamini–

Hochberg and the more conservative Benjamini–Yekutieli procedure, the latter valid under arbitrary dependence (m = 20; FDR threshold $q < 0.05$). Nominal p-values and adjusted q-values are reported together in Table 2. This analysis is exploratory and only nominates candidates for experimental screening. It is not interpreted as evidence of metabolic AA demand, which codon usage and translational biases may also affect [18].

2.7. Determination of Intracellular Acetyl-CoA Content

Fresh mycelia were collected at 0, 12, 24, 48, and 72 h of fermentation by vacuum filtration. The samples were immediately frozen in liquid nitrogen and ground into a powder. One gram of the powder was suspended in 3 mL of normal saline and mixed thoroughly. The mixture was centrifuged at 6000 rpm and 4 °C for 10 min, and the supernatant was filtered through a 0.22 μm membrane before analysis. Acetyl-CoA was measured using a commercial Mouse Acetyl-CoA ELISA kit from Shanghai Yuduo Biotechnology Co., Ltd. (Shanghai, China; Lot No. 202203) according to the manufacturer's instructions. The kit uses a one-step sandwich ELISA with absorbance measured at 450 nm. The supplied standards were 15, 30, 60, 120, and 240 ng/mL, with sensitivity < 1.0 ng/mL. For each sample well, 10 μL of sample was mixed with 40 μL of sample diluent for a five-fold dilution. Three independent flasks were analyzed for each treatment and sampling time. For Equation (2), the dilution-corrected extract concentration was converted from ng/mL to μg/mL and multiplied by the 3 mL extraction volume; the denominator was 1 g fresh mycelial powder.

$$\text{Intracellular acetyl} - \text{CoA content}(\mu g/g) = \frac{\text{Acetyl} - \text{CoA concentration}(\mu g/mL) \times \text{solvent volume}(3mL)}{\text{Weight of mycelial powder}(g)} \quad (2)$$

2.8. Statistical Analysis

All experiments were performed in biological triplicate ($n = 3$), and data are expressed as mean ± standard deviation (SD). Normality of the data was assessed by the Shapiro–Wilk test ($\alpha = 0.05$). Normally distributed data were analyzed by one-way ANOVA followed by Tukey's HSD post hoc test. Non-normally distributed data were analyzed by the Kruskal–Wallis test with Dunn's post hoc test. Different lowercase letters above bars denote significant differences at $p < 0.05$ according to the corresponding post hoc test. All statistical analyses were carried out with SPSS Statistics 26 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. AA Utilization Under High-Temperature Fermentation in S. bambusicola GDMCC 60438

To identify metabolic features associated with enhanced HA biosynthesis under high-temperature fermentation, the transcriptomic dataset previously reported by our group [4] was reanalyzed. The original RNA-seq data were obtained from *S. bambusicola* GDMCC 60438 cultivated at 26, 28, and 32 °C under identical fermentation conditions. No additional sequencing was performed in the present work; instead, the published dataset was reexamined to characterize AA utilization, including transporter expression and AA demand.

Since AA uptake is the first step linking extracellular AAs to intracellular metabolism, genes encoding AA transporters were analyzed first. AAPs and MFS transporters are the two major transporter families responsible for cellular AA uptake and are known to respond to environmental and nutritional signals [17,19,20]. Transcriptomic reanalysis showed that seven AAP genes and nine MFS transporter genes were significantly upregulated at 32 °C compared with both 26 and 28 °C (Table 1). Among them, *MH01c23g0116981*, encoding an AAP, was identified as a hub gene in the co-expression network of high-temperature-responsive genes previously constructed [4]. These results indicate that elevated temperature is associated with increased transcription of AA transporter genes, suggesting an enhanced potential for AA uptake during high-temperature fermentation.

Table 1. Pfam functional annotation of AA transporter genes highly expressed at 32 °C *.

Gene ID	Pfam ID	Pfam Name	Description
MH01c07g0049611	PF00324.20	AA_permease	AAP
MH01c20g0103751			
MH01c23g0116981			
MH01c01g0006851	PF13520.5	AA_permease_2	AAP
MH01c01g0008751			
MH01c03g0023741			
MH01c08g0055531			
MH01c02g0009511	PF07690.15	MFS_1	Major facilitator superfamily
MH01c02g0012091			
MH01c03g0018551			
MH01c04g0030501			
MH01c04g0032541			
MH01c07g0052031			
MH01c12g0077731			
MH01c13g0080241			
MH01c23g0116941			

* Genes highly expressed at 32 °C relative to both 26 °C and 28 °C (Cluster 1 of the hierarchical clustering described in Section 2.5 and ref. [4]). The table lists gene identifiers and Pfam annotations; expression statistics are available in the previously published dataset [4]. Pfam hits were retained at E-value $\leq 10^{-5}$.

To determine whether high temperature also altered intracellular AA utilization, the AA composition of proteins encoded by genes significantly upregulated at 32 °C was compared with that of the whole predicted proteome. Eight AAs showed a higher mean relative frequency in the 32 °C upregulated set than in the predicted proteome at an unadjusted two-sided $p < 0.05$; seven of these remained significant after false-discovery-rate correction (Gly, Ile, Lys, Phe, Trp, Tyr, and Val), whereas the nominal enrichment of Ala was not retained (Table 2; Figure 1). Such nominal enrichment indicates that proteins encoded by genes highly expressed at 32 °C possess a distinct AA composition, which is consistent with—but does not demonstrate—an altered requirement for these AAs.

Table 2. Differences in AA composition between proteins encoded by genes highly expressed at 32 °C and the whole-genome predicted proteome of *S. bambusicola* GDMCC 60438.

Amino Acid	Abbreviation	32 °C Upregulated Genes vs. All Genes	Nominal *p*-Value (Unadjusted)	BH *q*-Value	BY *q*-Value
Ala	A	Enriched	0.0452	0.065	0.232
Arg	R	Depleted	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Asn	N	Not significant	0.3446	0.383	1.000
Asp	D	Not significant	0.1211	0.162	0.581
Cys	C	Depleted	0.0271	0.042	0.150
Gln	Q	Depleted	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$

Table 2. *Cont.*

Amino Acid	Abbreviation	32 °C Upregulated Genes vs. All Genes	Nominal *p*-Value (Unadjusted)	BH *q*-Value	BY *q*-Value
Glu	E	Not significant	0.4176	0.440	1.000
Gly	G	Enriched	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
His	H	Depleted	1.8×10^{-5}	3.6×10^{-5}	1.3×10^{-4}
Ile	I	Enriched	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Leu	L	Not significant	0.6002	0.600	1.000
Lys	K	Enriched	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Met	M	Not significant	0.3196	0.376	1.000
Phe	F	Enriched	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Pro	P	Depleted	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Ser	S	Depleted	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$
Thr	T	Not significant	0.1318	0.165	0.593
Trp	W	Enriched	0.0020	0.0034	0.012
Tyr	Y	Enriched	1.9×10^{-4}	3.5×10^{-4}	1.2×10^{-3}
Val	V	Enriched	<0.001	$<1 \times 10^{-7}$	$<1 \times 10^{-7}$

Values are reported as nominal (unadjusted) two-sided *p*-values, as Benjamini–Hochberg (BH) adjusted *q*-values, and as Benjamini–Yekutieli (BY) adjusted *q*-values ($m = 20$). Enrichment reflects the AA composition of the encoded proteins and is not a direct measure of physiological AA demand. Adjusted *q*-values are given to three significant figures; for the nine comparisons whose unadjusted *p*-value was below the reporting precision of the original analysis ($<5 \times 10^{-9}$), the adjusted *q*-value is bounded above by 1.1×10^{-8} (BH) and 4.0×10^{-8} (BY) and is therefore reported as $<1 \times 10^{-7}$.

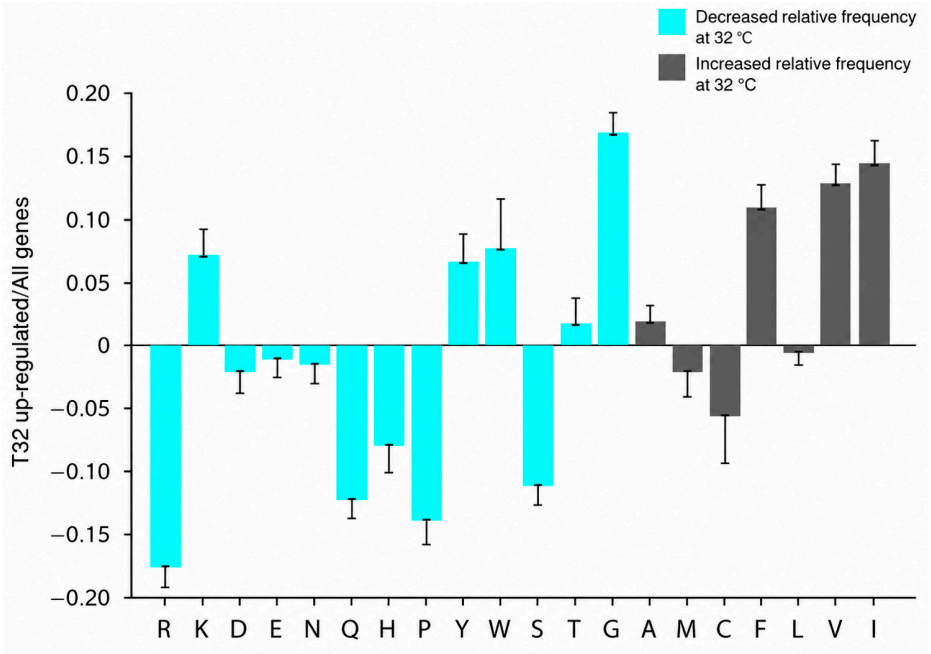


Figure 1. Relative differences in AA composition between proteins encoded by genes upregulated at 32 °C and the whole-genome predicted proteome of *S. bambusicola* GDMCC 60438. Cyan bars indicate amino acids with lower relative frequencies in proteins encoded by genes upregulated at 32 °C than in the whole-genome predicted proteome, whereas gray bars indicate amino acids with higher relative frequencies.

The two lines of transcriptomic evidence carry different analytical weight. The coordinated upregulation of AAP- and MFS-encoding genes provides evidence of altered transporter-related transcription at 32 °C—consistent with, but not a direct measurement of, an enhanced cellular capacity for AA uptake. In contrast, AA enrichment in highly expressed proteins may partly reflect codon usage bias and translational optimization rather than a direct metabolic demand [18] and is therefore treated as supporting evidence. When considered together, these two observations consistently suggest that high-temperature fermentation is accompanied by a substantial remodeling of AA utilization.

On the basis of these transcriptomic features, it was hypothesized that the increased physiological demand for specific AAs could be exploited through exogenous supplementation. Among the eight AAs showing nominal enrichment at 32 °C, five (Ala, Lys, Gly, Val, and Ile) were selected as candidates for subsequent experimental validation according to their known metabolic connectivity to central carbon metabolism and the acetyl-CoA/pyruvate node that supplies polyketide biosynthesis. These five candidates were therefore selected to evaluate their capacity to further enhance HA biosynthesis under high-temperature fermentation.

3.2. Transcriptome-Guided Screening Reveals AAs That Enhance HA Biosynthesis

The five candidate AAs (Ala, Lys, Gly, Val, and Ile) selected above were experimentally validated at 32 °C, at which intracellular HA content is maximal in *S. bambusicola* GDMCC 60438 [4].

Among the five AAs tested, Ala, Lys, and Gly significantly enhanced intracellular HA content without substantially affecting fungal growth at their optimal concentrations (Figure 2). Supplementation with 50 mg/L Ala increased intracellular HA content by 71% relative to the control, while mycelial biomass remained unchanged (Figure 2A). Lys exhibited the strongest stimulatory effect among the single AAs, with 200 mg/L increasing intracellular HA content by 74% (Figure 2B). However, Lys concentrations above 800 mg/L markedly inhibited mycelial growth, indicating toxicity at excessive supplementation levels. Gly supplementation at 100 mg/L enhanced intracellular HA content by 36% without affecting biomass, whereas concentrations above 200 mg/L gradually suppressed fungal growth (Figure 2C). In contrast, Val had no significant effect on intracellular HA content across the tested concentration range (all treatments $p > 0.05$ versus the control), whereas Ile produced only a marginal increase (approximately 15–20% at 400 mg/L; $p > 0.05$ versus the control) that did not reach statistical significance (Figure 2D,E).

These results indicate that only a subset of transcriptome-selected AAs functioned as effective metabolic elicitors and that transcriptomic enrichment alone was insufficient to predict the regulatory strength of individual AAs. Experimental validation therefore remained essential for identifying physiologically responsive AAs under high-temperature fermentation.

To further examine potential interactions among the candidate AAs, the concentrations selected in Section 2.3 were combined pairwise. Three binary combinations significantly enhanced intracellular HA content, namely Ala + Lys (+ 71%), Ile + Gly (+ 75%), and Val + Gly (+80%) (Figure 2F), and two distinct interaction patterns were observed. The Ala + Lys combination produced an increase comparable to that achieved by either AA alone; this pattern is consistent with, but does not formally demonstrate, convergence of Ala and Lys on a common downstream process, as no interaction analysis was performed. By contrast, Val + Gly resulted in the strongest enhancement observed in the present study, despite Val alone showing no statistically significant stimulatory effect across the entire concentration range tested (0–1000 mg/L; all $p > 0.05$ versus the control). The combined treatment exceeded the effect of Gly alone at the same concentration (100 mg/L),

whereas Val alone (400 mg/L) showed no significant effect. However, this comparison was not subjected to a formal interaction analysis, and the effect is therefore described as an enhanced combined effect rather than as demonstrated synergy. This enhanced combined effect is suggestive of a metabolic interaction between Val and Gly that merits further investigation using an appropriately designed factorial experiment.

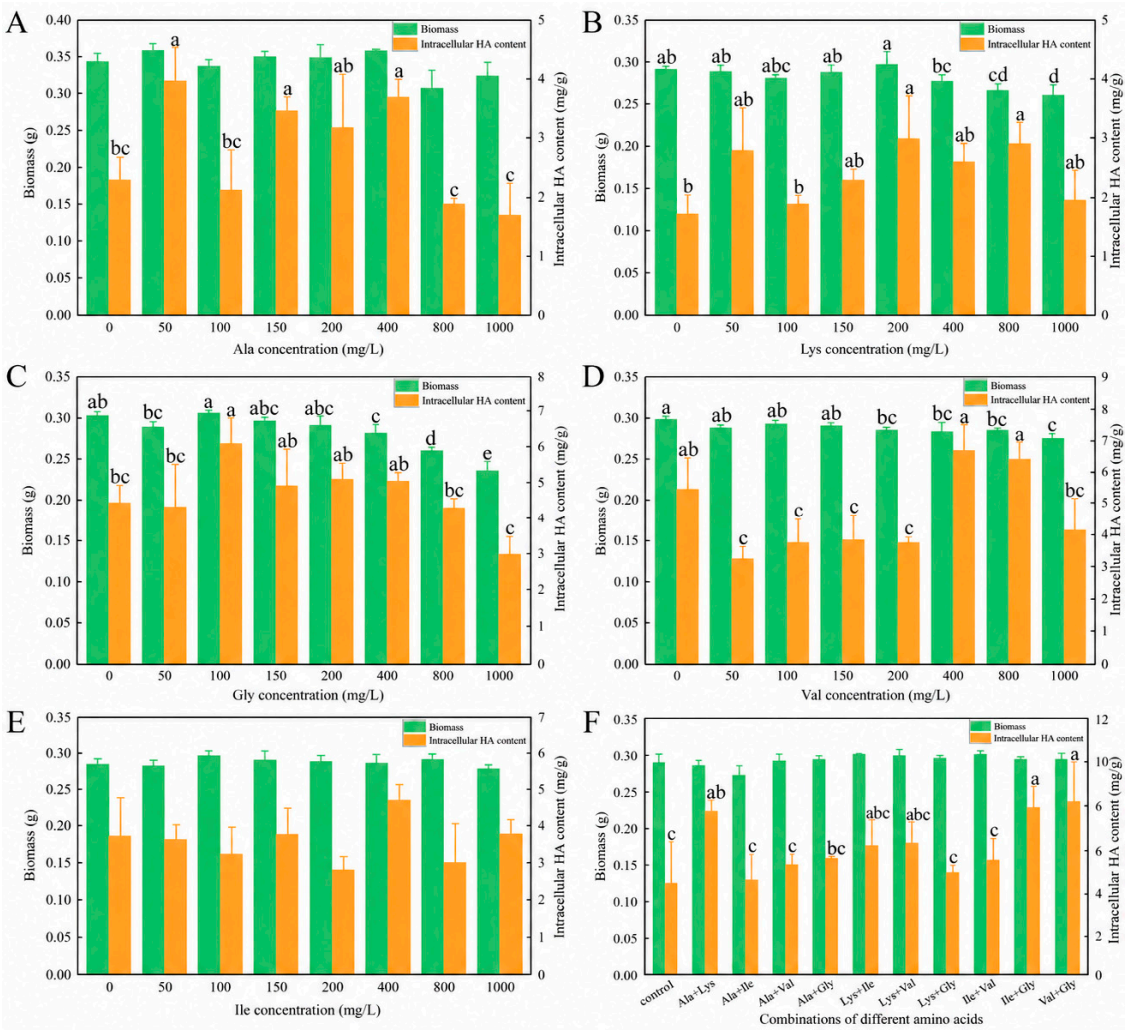


Figure 2. Effects of exogenous AA supplementation on biomass and intracellular HA content in mycelium of S. bambusicola GDMCC 60438 at 32 °C: Effects of (**A**) Ala (**B**) Lys (**C**) Gly (**D**) Val and (**E**) Ile supplementation at 0, 50, 100, 150, 200, 400, 800, and 1000 mg/L on mycelial biomass and intracellular HA content, respectively; (**F**) effects of pairwise AA combinations of Ala (50 mg/L), Lys (200 mg/L), Gly (100 mg/L), Val (400 mg/L), and Ile (400 mg/L) on mycelial biomass and intracellular HA content; the control contained no added AA. Different letters above bars indicate significant differences at $p < 0.05$ within each panel (Tukey's HSD, $n = 3$).

Previous studies have shown that coordinated AA metabolism can influence fungal polyketide biosynthesis by regulating precursor supply and central carbon metabolism [12,13]. In particular, branched-chain amino acids (BCAAs) such as Val, Leu, and Ile have been reported to elicit perylenequinone production in *Shiraia* mycelium cultures, where their catabolic products enter central carbon metabolism and modulate downstream secondary-metabolite flux [6]. Given that Val alone did not stimulate intracellular HA content across the tested concentration range, the combined effect of Val and Gly raises the possibility that the two AAs affect complementary aspects of metabolism. This interpretation remains hypothetical and requires validation by factorial interaction analysis.

Whether the stimulatory effects observed were specific to the high-temperature physiological state was next evaluated by comparing AA supplementation under conventional (28 °C) and high-temperature (32 °C) fermentation.

3.3. AA Supplementation Responses at 28 and 32 °C

To determine whether the stimulatory effects of AA supplementation reflected a general nutritional response or were specifically associated with high-temperature fermentation, the three most effective AAs identified at 32 °C (Ala, Lys, and Gly) were further evaluated at the conventional fermentation temperature of 28 °C.

None of the three AAs significantly affected mycelial biomass at 28 °C (Figure 3). Lys supplementation increased intracellular HA content by 44% relative to the control, whereas Ala and Gly produced only modest increases of 11% and 20%, respectively. Compared with the corresponding effects at 32 °C, all three AAs exhibited substantially weaker stimulation of HA biosynthesis at 28 °C.

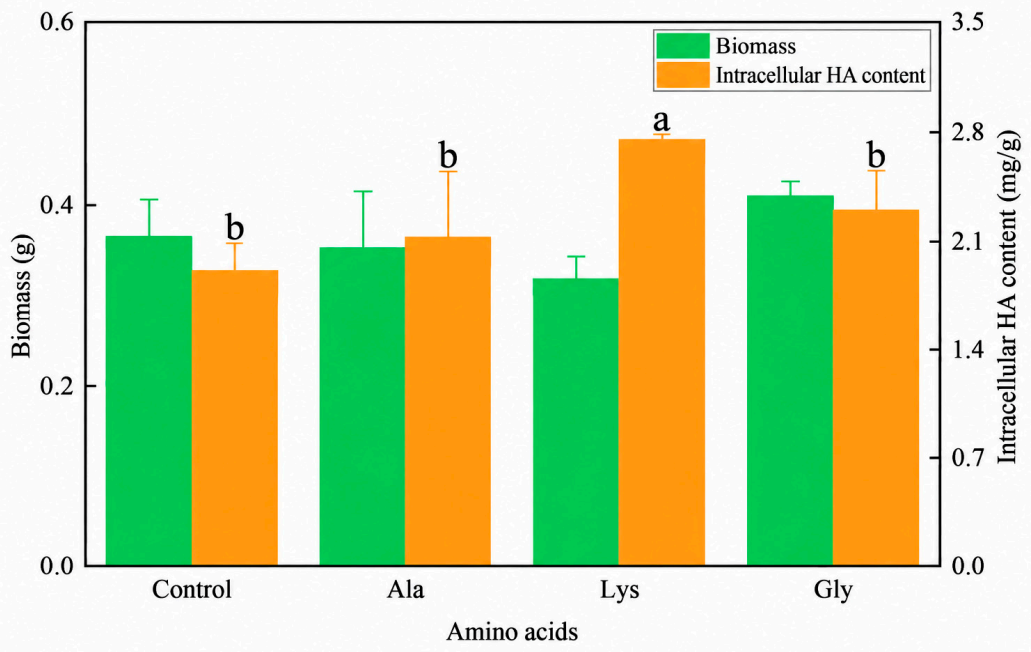


Figure 3. Effects of exogenous AA supplementation on biomass and intracellular HA content of *S. bambusicola* GDMCC 60438 at 28 °C. The four groups (Control, Ala, Lys, and Gly) correspond to the fermentation medium without supplementation and to media supplemented with Ala (50 mg/L), Lys (200 mg/L), or Gly (100 mg/L) at the optimal concentrations identified at 32 °C. Values are expressed as mean ± SD ($n = 3$). Different lowercase letters above bars indicate significant differences at $p < 0.05$ (Tukey's HSD).

These separate experiments show a larger observed response magnitude to Ala, Lys, and Gly at 32 °C than at 28 °C. Temperature and AA treatment were not analyzed in a common factorial model, and no temperature × AA interaction was tested. The 28 °C and 32 °C experiments were conducted as independent screening experiments, while the binary-combination experiment was performed only at 32 °C. Pooling these separate batches could confound the temperature effect with batch-to-batch variation. The data do not statistically demonstrate temperature dependence. The AA response is therefore interpreted as being associated with a high-temperature physiological state while remaining provisional. The subsequent acetyl-CoA experiment therefore focused on Ala, Lys, and Gly at 32 °C as a preliminary test of a possible metabolic association.

3.4. AA Supplementation Is Associated with Early Changes in Acetyl-CoA Content in Mycelium

To examine a possible metabolic link between AA supplementation and enhanced HA biosynthesis, intracellular acetyl-CoA levels were monitored during fermentation in cultures supplemented with Ala, Lys, or Gly. Acetyl-CoA is the universal precursor for fungal polyketide biosynthesis, including HA, and thus represents a plausible downstream metabolic node connecting AA metabolism with HA production [14,15].

Dynamic profiling revealed distinct temporal changes in acetyl-CoA in mycelium of *S. bambusicola* (Figure 4). During the initial 0–12 h of fermentation, acetyl-CoA concentrations increased in cultures supplemented with Ala, Lys, or Gly, whereas the control culture exhibited a continuous decline. Between 12 and 24 h, acetyl-CoA levels decreased sharply across all the treatment groups. Because the data were analyzed as separate exploratory comparisons at each time point, without cross-time multiplicity correction or a treatment × time interaction test, and because growth phase, metabolic flux, and secondary-metabolism activity were not independently characterized, the underlying physiological basis cannot be determined from the current measurements alone.

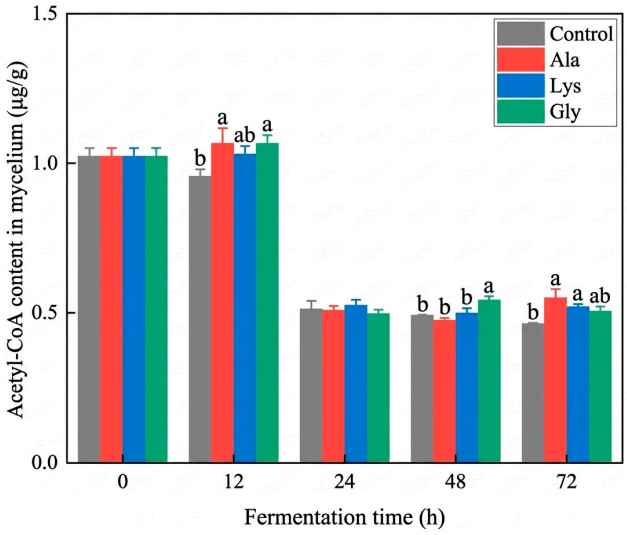


Figure 4. Dynamic profile of acetyl-CoA content in mycelium of *S. bambusicola* GDMCC 60438 during supplementation with Ala, Lys, or Gly at 32 °C. Values are mean ± SD (n = 3 independent flasks per treatment and time point). At each sampling time, different letters indicate significant differences among treatments at $p < 0.05$ (Tukey's HSD). Five separate time-point analyses were performed without cross-time multiplicity adjustment, and no treatment × time interaction was tested.

The elevated acetyl-CoA levels observed during the early fermentation stage are consistent with the established catabolic pathways of AAs, in which Ala and Gly are converted into pyruvate and subsequently acetyl-CoA, whereas Lys is degraded via acetoacetyl-CoA before entering central carbon metabolism [21]. Although these data do not directly demonstrate carbon flux from AAs into HA biosynthesis, they indicate that AA supplementation may transiently expand the intracellular acetyl-CoA pool available for polyketide biosynthesis.

Overall, these observations support acetyl-CoA as a plausible downstream metabolic node linking AA utilization with enhanced HA biosynthesis under high-temperature fermentation. Acetyl-CoA dynamics were measured only for the three effective single AAs (Ala, Lys, and Gly); therefore, the metabolic basis of the binary combinations, particularly the pronounced Val + Gly combined effect, cannot be resolved from the present dataset and will require dedicated analysis of AA combinations in future work.

3.5. Working Model for the Stronger AA Response Observed at 32 °C

Based on the transcriptomic, physiological, and metabolic evidence obtained in the present study, a working model is proposed to explain the enhanced responsiveness of *S. bambusicola* to exogenous AA supplementation during high-temperature fermentation (Figure 5).

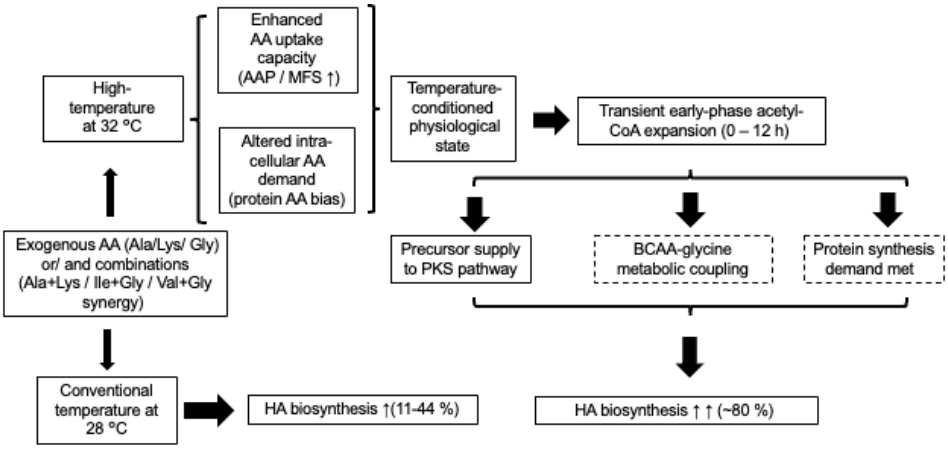


Figure 5. Working model for the stronger AA-supplementation response observed at 32 °C in *S. bambusicola*. Solid arrows indicate experimentally observed temporal or treatment associations in the present study without implying direct causality. Dashed arrows indicate proposed mechanistic links that require validation by transporter assays, factorial interaction tests, isotope tracing, or metabolic-flux analysis. The upward arrow (↑) indicates a proposed increase in AA uptake capacity associated with the upregulation of AA permease (AAP) and major facilitator superfamily (MFS) transporter genes at 32 °C.

Relative to 28 °C, fermentation at 32 °C was associated with coordinated upregulation of AA transporter genes, an altered AA composition of proteins encoded by highly expressed genes, and enhanced responsiveness to exogenous AA supplementation. These observations indicate that high-temperature fermentation establishes a distinct physiological state favorable for AA elicitation.

Within this physiological context, exogenous AAs may be more efficiently assimilated into central metabolism and protein biosynthesis. AA catabolism may also transiently expand the intracellular acetyl-CoA pool, thereby increasing precursor availability for HA biosynthesis. Such mechanisms provide a plausible explanation for the markedly stronger stimulatory effects of AA supplementation observed at 32 °C.

The proposed model integrates transcriptomic reanalysis, AA supplementation, and intracellular acetyl-CoA dynamics into a unified framework describing temperature-associated AA elicitation of HA biosynthesis. Although several mechanistic links remain hypothetical, this framework provides a basis for further experimental validation and rational fermentation optimization.

4. Discussion

Temperature is a critical environmental factor controlling fungal growth, development, and secondary metabolism. Previous studies on *S. bambusicola* have shown that elevated fermentation temperature significantly promotes HA accumulation through coordinated regulation of mycelial morphology, transporter expression, and biosynthetic gene activation [4]. However, the metabolic basis underlying this temperature-associated enhancement has remained unclear. In the present study, the stimulatory effects of AA supplementation were more pronounced at 32 °C, with Ala, Lys, and Gly showing substantially greater effects than at 28 °C. As described in Section 3.3, the two temperatures were evaluated

in separate experiments, and a formal temperature × AA interaction was not tested. The difference is therefore interpreted as a larger observed response magnitude rather than a statistically demonstrated temperature dependence.

Temperature-responsive regulation of fungal secondary metabolism is often mediated by global regulatory networks that integrate environmental signals with cellular metabolic status. For instance, the Velvet regulatory complex in Aspergillus coordinates secondary metabolism, development, and environmental adaptation, and its activity is strongly influenced by temperature-associated signals [11]. Related regulatory reprogramming has been documented in other filamentous fungi, in which environmental stress triggers transcriptional remodeling and redistribution of cellular resources toward secondary metabolite production [13,22,23]. Consistent with these findings, the transcriptomic reanalysis performed in the present study revealed coordinated upregulation of AA transporter genes at 32 °C, including AAP- and MFS-encoding genes. This result supports altered transporter-related transcription at 32 °C. The exploratory AA-composition analysis similarly suggests an association with the 32 °C transcriptional program. Although transporter expression alone does not directly demonstrate increased AA flux, the combined evidence supports a working hypothesis that the high-temperature physiological state may influence AA responsiveness.

AAs have increasingly been recognized as regulatory molecules involved in fungal secondary metabolism rather than mere nitrogen sources. Different AAs may exert distinct or even opposite effects depending on fungal species and metabolic context. For example, AA supplementation differentially regulates pigment and citrinin biosynthesis in *Monascus*, with some AAs promoting one pathway while repressing another [23], while studies in *Shiraia* have shown that nutrient status, elicitors, and BCAA supplementation can substantially alter perylenequinone production [6,7,22]. In the present study, transcriptome-guided selection identified Ala, Lys, Gly, Val, and Ile as candidate AAs, but only a subset effectively enhanced HA biosynthesis. This discrepancy indicates that AA responsiveness cannot be predicted solely by abundance or transcriptional association but rather depends on the metabolic state of the producing cells. The strong enhancement achieved by Val + Gly, despite the limited effect of Val alone, is compatible with an interaction between the two treatments but does not establish its underlying mechanism.

The enhancement effect of AA combinations raises a mechanistic question: how do different AA metabolic pathways converge to regulate HA biosynthesis? One possibility is that AAs regulate secondary metabolism through coordinated effects on central carbon metabolism and precursor availability. Acetyl-CoA is a key precursor for fungal polyketide biosynthesis, including HA, and AA supplementation may influence acetyl-CoA availability through the regulation of central carbon metabolism and precursor supply [24]. Previous work in oleaginous microorganisms has shown that AA metabolism can influence acetyl-CoA availability and lipid biosynthesis, highlighting the close relationship between AA catabolism and central carbon metabolism [16]. In agreement with these findings, AA supplementation increased intracellular acetyl-CoA levels during the early fermentation stage in the present study. This observation is compatible with a precursor-availability mechanism but does not establish carbon transfer from the supplemented AAs into HA. Moreover, acetyl-CoA was not measured after binary supplementation; therefore, no conclusion can be drawn about the metabolic basis of the Val + Gly, Ile + Gly, or Ala + Lys combination effects. Additional mechanisms, including nitrogen regulation, translational demand, and metabolic signaling, may contribute to the temperature-associated response.

Compared with conventional empirical medium optimization, the strategy developed here provides a transcriptome-guided approach for identifying condition-specific fermentation regulators. Previous efforts to improve HA production have relied mainly on strain

engineering [25–27], gamma-ray or ultrasonic mutagenesis [28,29], signal transduction manipulation and co-culture [30,31], and nutrient optimization and chemical elicitation [6,7], and these approaches generally identify effective interventions through extensive screening. By contrast, our approach used a defined environmental perturbation and the associated transcriptomic response to nominate AA candidates before experimental validation. This strategy therefore constitutes a transcriptome-informed approach to fermentation optimization that may prove useful for enhancing the accumulation of related secondary metabolites; however, as it was evaluated in a single strain and for a single metabolite, further validation in other biological systems is required before general applicability can be established.

The data support a model in which high-temperature fermentation enhances AA responsiveness through coordinated changes in transport, cellular demand, and precursor metabolism, but the causal relationships remain to be established. Future work should directly measure AA uptake, apply temperature × AA and AA × AA factorial designs, and integrate stable-isotope tracing with metabolomics or metabolic-flux analysis. Such experiments would determine whether the observed response reflects altered precursor routing, regulatory signaling, translational demand, or a combination of these mechanisms. Nevertheless, the present study provides evidence that temperature-associated AA elicitation represents an effective process-level strategy for enhancing HA production under the tested condition and offers a rational framework for transcriptome-guided fermentation optimization.

5. Conclusions

High-temperature fermentation was associated with larger observed responses of intracellular HA content to targeted AA supplementation in *S. bambusicola*. At 32 °C, single supplementation with Ala, Lys, or Gly, and binary combinations (Ala + Lys, Ile + Gly, Val + Gly), significantly improved intracellular HA content, whereas single supplementation with Ala, Lys, or Gly produced markedly weaker effects at 28 °C. Overall, this study not only provides a transcriptome-guided basis for selecting AA supplements at 32 °C and defines the factorial and mechanistic experiments required for confirmation, but also, through the transcriptomic reanalysis together with the dynamic profile of intracellular acetyl-CoA, links temperature-associated AA induction to enhanced HA biosynthesis, thereby offering a transcriptome-guided starting point for optimizing fungal secondary metabolite production.

Author Contributions: Conceptualization, Y.W. and X.T.; methodology, Y.W.; investigation, Y.W. and Q.W.; resources, X.T.; data curation, Y.W. and Q.W.; writing—original draft preparation, Q.W., J.L. and Y.W.; writing—review and editing, X.T. and F.Y.; supervision, X.T. and Z.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundamental Research Funds for the Central Universities (grant number 2025ZYGXZR104) and the Yunnan Key Laboratory for Fungal Diversity and Green Development, China (grant number 202205AG070092).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting the fermentation experiments are contained within the article. The transcriptomic dataset reanalyzed here was published in Reference [4] (https://doi.org/10.1186/s12934-022-01862-w; accessed on 4 October 2026). Gene-level TPM values are provided in Tables S2 and S4 of the Supplementary Information of that publication, and no separate accession number was reported in the original article.

Acknowledgments: The authors would like to thank Lining Wang, Institute of Biological and Medical Engineering, Guangdong Academy of Sciences, China, for technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

HA, hypocrellin A; AA, amino acid; AAP, amino acid permease; MFS, major facilitator superfamily; PKS, polyketide synthase; HPLC, high-performance liquid chromatography; PDA, potato dextrose agar; PDB, potato dextrose broth.

References

1. Zhang, X.; Wei, Q.; Tian, L.; Huang, Z.; Tang, Y.; Wen, Y.; Yu, F.; Yan, X.; Zhao, Y.; Wu, Z.; et al. Advancements and Future Prospects in Hypocrellins Production and Modification for Photodynamic Therapy. *Fermentation* **2024**, *10*, 559. [CrossRef]
2. Huang, J.; Law, S.K.; Leung, A.W.; Xu, C. Photodynamic Action of Hypocrellin A and Hypocrellin B against Cancer—A Review. *Pharmaceuticals* **2025**, *18*, 1847. [CrossRef]
3. Li, X.P.; Shen, W.H.; Wang, J.W.; Zheng, L.P. Production of Fungal Hypocrellin Photosensitizers: Exploiting Bambusicolous Fungi and Elicitation Strategies in Mycelium Cultures. *Mycology* **2025**, *16*, 593–616. [CrossRef]
4. Wen, Y.; Liao, B.; Yan, X.; Wu, Z.; Tian, X. Temperature-Responsive Regulation of the Fermentation of Hypocrellin A by *Shiraia bambusicola* (GDMCC 60438). *Microb. Cell Fact.* **2022**, *21*, 135. [CrossRef]
5. Bao, Z.; Xie, Y.; Zhu, D. Biotechnological Production and Potential Applications of Hypocrellins. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 6421–6438. [CrossRef]
6. Shen, W.H.; Cong, R.P.; Li, X.P.; Huang, Q.Y.; Zheng, L.P.; Wang, J.W. Effects of Branched-Chain Amino Acids on *Shiraia* Perylenequinone Production in Mycelium Cultures. *Microb. Cell Fact.* **2023**, *22*, 57. [CrossRef]
7. Shen, W.H.; Zhou, L.L.; Li, X.P.; Cong, R.P.; Huang, Q.Y.; Zheng, L.P.; Wang, J.W. Bamboo Polysaccharides Elicit Hypocrellin A Biosynthesis of a Bambusicolous Fungus *Shiraia* sp. S9. *World J. Microbiol. Biotechnol.* **2023**, *39*, 341. [CrossRef]
8. Yan, X.; Wen, Y.; Hu, M.; Wu, Z.; Tian, X. Promotion of the Hypocrellin Yield by a Co-Culture of *Shiraia bambusicola* (GDMCC 60438) with *Arthrinium* sp. AF-5 Fungus. *Fermentation* **2021**, *7*, 316. [CrossRef]
9. Tang, Y.; Wen, Y.; Zhang, X.; Gao, Q.; Yu, F.; Wu, Z.; Tian, X. Urea-Induced Enhancement of Hypocrellin A Synthesis in *Shiraia bambusicola* GDMCC 60438: Strategies and Mechanisms. *Fermentation* **2024**, *10*, 381. [CrossRef]
10. Zhang, X.; Wei, Q.; Tang, Y.; Yu, F.; Wu, Z.; Tian, X. Microparticle-Enhanced Cultivation of *Shiraia bambusicola* GDMCC 60438 Improves Hypocrellin A Production via SiO_2-Induced Pellet Remodeling and Oxidative Stress. *Fermentation* **2026**, *12*, 163. [CrossRef]
11. Lind, A.L.; Smith, T.D.; Saterlee, T.; Calvo, A.M.; Rokas, A. Regulation of Secondary Metabolism by the Velvet Complex Is Temperature-Responsive in *Aspergillus*. *G3 Genes Genomes Genet* **2016**, *6*, 4023–4033. [CrossRef]
12. Wang, J.; Zhang, R.; Chen, X.; Sun, X.; Yan, Y.; Shen, X.; Yuan, Q. Biosynthesis of Aromatic Polyketides in Microorganisms Using Type II Polyketide Synthases. *Microb. Cell Fact.* **2020**, *19*, 110. [CrossRef]
13. Keller, N.P. Fungal Secondary Metabolism: Regulation, Function and Drug Discovery. *Nat. Rev. Microbiol.* **2019**, *17*, 167–180. [CrossRef]
14. Zhao, N.; Li, D.; Guo, B.J.; Lin, X.; Qi, S.S.; Yan, S.Z. Genome Sequencing and Analysis of the Hypocrellin-Producing Fungus *Shiraia bambusicola* S4201. *Front. Microbiol.* **2020**, *11*, 643. [CrossRef]
15. Ning, Z.; Lin, X.; Qi, S.S.; Luo, Z.M.; Chen, S.L.; Yan, S.Z. De Novo Transcriptome Assembly in *Shiraia bambusicola* to Investigate Putative Genes Involved in the Biosynthesis of Hypocrellin A. *Int. J. Mol. Sci.* **2016**, *17*, 311. [CrossRef]
16. Takagi, H. Metabolic Regulatory Mechanisms and Physiological Roles of Functional Amino Acids and Their Applications in Yeast. *Biosci. Biotechnol. Biochem.* **2019**, *83*, 1449–1459. [CrossRef]
17. Bianchi, F.; Van't Klooster, J.S.; Ruiz, S.J.; Poolman, B. Regulation of Amino Acid Transport in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **2019**, *83*, e00024-19. [CrossRef]
18. Parvathy, S.T.; Udayasuriyan, V.; Bhadana, V. Codon Usage Bias. *Mol. Biol. Rep.* **2022**, *49*, 539–565. [CrossRef]
19. Deng, H.; Gao, R.; Chen, J.; Liao, X.; Cai, Y. Characterization of a Major Facilitator Superfamily Transporter in *Shiraia bambusicola*. *Res. Microbiol.* **2017**, *168*, 664–672. [CrossRef]
20. Yang, Y.; Li, Y.; Zhu, J. Research Progress on the Function and Regulatory Pathways of Amino Acid Permeases in Fungi. *World J. Microbiol. Biotechnol.* **2024**, *40*, 392. [CrossRef]
21. Nelson, D.L.; Cox, M.M. *Lehninger Principles of Biochemistry*, 7th ed.; W.H. Freeman: New York, NY, USA, 2017; pp. 693–726.
22. Wang, G.; Ran, H.; Fan, J.; Keller, N.P.; Liu, Z.; Wu, F. Fungal-Fungal Cocultivation Leads to Widespread Secondary Metabolite Alteration Requiring the Partial Loss-of-Function VeA1 Protein. *Sci. Adv.* **2022**, *8*, eabo6094. [CrossRef]

23. Chen, Y.; Wang, Y.; Li, X.; Wang, C.; Zhang, X.; Chen, M. Diverse Effects of Amino Acids on *Monascus* Pigments Biosynthesis in *Monascus purpureus*. *Front. Microbiol.* **2022**, *13*, 951266. [CrossRef]
24. Yu, W.; Pei, R.; Zhou, J.; Zeng, B.; Tu, Y.; He, B. Molecular Regulation of Fungal Secondary Metabolism. *World J. Microbiol. Biotechnol.* **2023**, *39*, 204. [CrossRef]
25. Deng, H.; Gao, R.; Chen, J.; Liao, X.; Cai, Y. Genome Editing in *Shiraia bambusicola* Using CRISPR-Cas9 System. *J. Biotechnol.* **2017**, *259*, 228–234. [CrossRef]
26. Li, D.; Zhao, N.; Guo, B.J.; Lin, X.; Chen, S.L.; Yan, S.Z. Genetic Overexpression Increases Production of Hypocrellin A in *Shiraia bambusicola* S4201. *J. Microbiol.* **2019**, *57*, 154–162. [CrossRef]
27. Gao, R.; Xu, Z.; Deng, H.; Guan, Z.; Liao, X.; Zhao, Y.; Zheng, X.; Cai, Y. Enhanced Hypocrellin Production of *Shiraia* sp. SUPER-H168 by Overexpression of the Alpha-Amylase Gene. *PLoS ONE* **2018**, *13*, e0196519. [CrossRef]
28. Liu, X.Y.; Shen, X.Y.; Fan, L.; Gao, J.; Hou, C.L. High-Efficiency Biosynthesis of Hypocrellin A in *Shiraia* sp. Using Gamma-Ray Mutagenesis. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 4875–4883. [CrossRef]
29. Sun, C.X.; Ma, Y.J.; Wang, J.W. Enhanced Production of Hypocrellin A by Ultrasound Stimulation in Submerged Cultures of *Shiraia bambusicola*. *Ultrason. Sonochem.* **2017**, *38*, 214–224. [CrossRef]
30. Liu, B.; Bao, J.Y.; Zhang, Z.B.; Yan, R.M.; Wang, Y.; Yang, H.L.; Zhu, D. Enhanced Production of Perylenequinones in the Endophytic Fungus *Shiraia* sp. Slf14 by Calcium/Calmodulin Signal Transduction. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 153–163. [CrossRef]
31. Ma, Y.; Zheng, L.; Wang, J. Inducing Perylenequinone Production from *Shiraia* sp. S9 through Co-Culture with *Pseudomonas fulva* SB1. *Microb. Cell Fact.* **2019**, *18*, 121. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.