ACTA SCIENTIAE

ISSN: 2178-7727

Genomic and Molecular Identification and PCR-Based Validation of the Fungal Luciferase Gene from Armillaria mellea Reveals Bioluminescence Genetic Potential

Akriti Singh[1], Parul Gautam[2], Aziz Mohammad Khan[3]*

Corresponding author e-mail: akriti.ak.1229@gmail.com
[1,2]Department of Food and Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur-303122, Rajasthan, India
[3*]Department of Food and Biotechnology, Faculty of Education and Methodology, Jayoti Vidyapeeth Women's University, Jaipur-303122, Rajasthan, India

ABSTRACT
Fungal bioluminescence is a genetically regulated biochemical phenomenon driven by a conserved luciferin-luciferase system. This study aimed to genomically identify and molecularly validate the fungal luciferase (Luz) gene from Armillaria mellea to confirm its inherent bioluminescent genetic potential. The fungal strain was cultured under standardized conditions on Malt Extract Agar and in broth media to obtain sufficient mycelial biomass for genomic DNA extraction. High-quality genomic DNA was isolated and used for sequencing library preparation followed by Illumina-based genome sequencing. Gene-specific primers were designed to amplify a conserved fragment of the Luz gene. PCR amplification yielded a distinct 785 bp amplicon corresponding to the expected size of the luciferase gene fragment. Agarose gel electrophoresis confirmed specific amplification without nonspecific bands or primer-dimer formation. The amplified product was further validated by Sanger sequencing, and BLASTn analysis demonstrated high sequence homology with previously reported fungal luciferase genes associated with the caffeic acid-dependent bioluminescence pathway. Sequencing chromatograms displayed clear nucleotide peaks, confirming high-quality sequence data.

KEYWORDS: Crew Lifestyle Monitoring, Artificial Intelligence, Fatigue Management, Wearable Technology, Aviation Safety, Health Analytics

INTRODUCTION
Armillaria mellea, which is commonly known as honey fungus, belongs to basidiomycete fungus within the family Physalacriaceae. The species is found throughout the temperate regions around the globe. It is considered to be of poor quality as it has a yellow-brown cap as well as long, rhizomorphous species that resemble a system of roots. The fungus is particularly notorious in that it is extremely causal, particularly on the roots of various woody plants, as well as trees. It can effectively cause the decay of the root node, whereby there were substantial economic losses that resulted from this type of infection within forestry and agriculture. Beyond its ecological and pathological significance, several species within the genus *Armillaria* are recognized for their intrinsic bioluminescence properties. *Armillaria mellea* has a wide array of applications, all of which depend on its pathological features. Although *Armillaria mellea* is a pathogen, it can also act as a saprophyte, thus serving as a potential source of organic decay in forest ecosystems. It feeds on dead trees, and, along with its wood-decomposing abilities, it can also help recalculate the nutrients back into the soil, thus benefiting the wild plants and trees. The primary bastion of any fungus is its fruiting body, the part we see above ground that produces basidiospores which are windblown long-distances to help the new, fresh colonization. *Armillaria mellea* also forms rhizomorphsthick, black, root-like structures that move nutrients and water between the substrate and mycelium, helping to keep the organism alive across unfavorable environments.

Fungal bioluminescence is a genetically regulated biochemical process involving a luciferin–luciferase system that produces visible green light. Recent advances have elucidated a caffeic acid–dependent bioluminescence pathway in basidiomycete fungi, consisting of a minimal gene cluster including luciferase (Luz), hispidin synthase (HispS), hispidin-3-hydroxylase (H3H), and caffeylpyruvate hydrolase (CPH) (Oba *et al*., 2017; Kotlobay *et al*., 2018). The luciferase enzyme (Luz) serves as the central catalytic component responsible for

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

light emission and represents a key molecular marker for confirming bioluminescent potential. The fungal bioluminescence pathway was comprehensively characterized through biochemical and genomic approaches in pioneering studies by Identification of the fungal luciferin pathway for autonomous luminescence in plants and genetically encodable bioluminescent system from fungi, which demonstrated that the pathway is genetically encodable and transferable across biological systems. These findings opened new avenues for synthetic biology, autonomous reporter systems, and luciferin-independent luminescent platforms. Advancements in next-generation sequencing technologies have further enabled genome-wide exploration of metabolic and secondary metabolite pathways in fungi (Bentley *et al*., 2008). De novo genome assembly and functional annotation provide essential insights into gene organization, pathway completeness, and evolutionary conservation. In luminous basidiomycetes, conservation of the bioluminescence gene cluster suggests a shared evolutionary origin and stable genomic integration (Oliveira *et al*., 2015; Kotlobay *et al*., 2018). Despite the growing understanding of fungal bioluminescence, genomic identification and molecular validation of the luciferase (Luz) gene in specific *Armillaria mellea* isolates remain limited, particularly in region-specific strains. Molecular confirmation of the Luz gene is essential to establish intrinsic bioluminescent genetic capability and to support downstream functional and comparative genomic studies.
Therefore, the present study aimed to genomically identify and PCR-validate the fungal luciferase (Luz) gene from *Armillaria mellea*, followed by sequence confirmation through Sanger sequencing and comparative BLAST analysis. This work provides molecular evidence supporting the presence of a conserved bioluminescence gene and establishes a genomic framework for future functional characterization and biotechnological exploitation of autonomous fungal light-emitting systems.

MATERIAL AND METHODS

Sample Collection and Culture Media Preparation

The strain *Armillaria mellea* was isolated from healthy mature basidiomata collected from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, from healthy adult basidiomata (Atlas 2010). For the cultivation of *Armillaria mellea* Malt Extract Agar (MEA) was the medium of choice. Malt extract agar medium was made by mixing 3.0 grams of malt extract powder, 3.0 grams of yeast extract powder, 5.0 grams of peptone powder, 10.0 grams of glucose, and 20.0 grams in 1000 mL of distilled water and the pH was set to 6.20 at 24^{o}C. In order to obtain a more developed culture of *Armillaria mellea* malt extract broth medium was used which was made by mixing 3.0 grams of malt extract powder, 3.0 grams of yeast extract powder, 5.0 grams of peptone powder, and 10.0 grams of glucose in 1000 mL of distilled water and the pH was set to 6.20 at 24^{o}C. Every from of media prepared was autoclaved at 121^{o}C for 15 minutes following standard microbiological procedures (Sambrook & Russell 2001).

Genomic DNA Extraction

Armillaria mellea samples were immediately freeze-dried to maintain the fungal tissue. The tissue was then ground into a fine powder with the aid of liquid nitrogen to rupture the cells for easy DNA extraction. Plant/Fungal DNA Miniprep Kit (Qiagen) was utilized to extract genomic DNA from the isolates which is a reliable method for obtaining high-quality plant and fungal genomes (Doyle and Doyle 1987). The DNA extraction process was conducted according to manufacturer guidelines to obtain the best yield. DNA yield was determined by NanoDrop spectrophotometer for analyzing DNA quality and quantity. DNA purity and concentration were estimated by measuring the absorbance of the sample using this tool.

Sequencing Library Preparation

The DNA was extracted in such a way that it could be sequenced. DNA was fragmented to provide the small pieces required for sequencing library preparation. These short fragments were then ligated to sequence adapters and amplified by PCR to be sequenced (Bentley *et al*., 2008). This library was sequenced as pairedends on the Illumina Novaseq 6000, a rapid sequencing technology that provides high-throughput data. The sequencing outputs 150 bp reads, which balances depth with resolution. Following library preparation, a Bioanalyzer was used for quality control of the fragmentation that showed an amplicon size of more than or equal to ~300 to 350 bp before sequencing.

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

Primer Design and Identification of Fungal bioluminescence Genes

Target genes responsible for fungal bioluminescence (i.e., Luciferase), H3H (hispidin-3-hydroxylase), CPH (caffeylpyruvate hydrolase), HispS (hispidin synthase), were identified and amplified using gene -specific designed primer. NCBI Primer-BLAST validated the designed primers for accuracy in gene amplification (Ye *et al*., 2012). GC content parameters were set between 40-60 %, along with melting temperature (Tm) in the range of 55-65ºC. Target forward and reverse genes Luz were 700-800 bp. Taxonomic reference data validated the target species as *Armillaria* (Taxonomy ID: 47429) in NCBI GenBank. To eliminate secondary structures like hairpins and primer-dimers, OligoAnalyzer (Integrated DNA Technology, IDT) was used. Primers were synthesized commercially.

PCR Amplification Protocol for the Luz Genes

Genomic DNA was isolated in accordance with the manufacturer's protocols to optimize the yield of DNA extraction. Isolated DNA was evaluated for both concentration and quality through the use of a NanoDrop spectrophotometer which employs absorbance ratios to determine the quality versus the quantity of DNA isolated (Sperschneider *et al*., 2015). To prepare the isolated DNA for downstream sequencing, the DNA was fragmented, and sequencing adapters were ligated and subsequently, the DNA was amplified through PCR to enrich for the desired fragments. PCR of the DNA was performed under a total reaction volume of 20 µL consisting of 2.0 µL of 10× PCR buffer, 0.6 µL of 2.5 mM dNTP mixture, 0.4 µL of a forward primer, 0.4 µL of a reverse primer, 0.1 µL of Taq DNA polymerase, 1.0 µL of template DNA, and 15.5 µL of nuclease free water. The cycling conditions were optimized according to primer melting temperatures and included an initial denaturation, followed by 30 to 35 amplification cycles, and a final extension step to ensure complete amplification of the target fragment.

Agarose Gel Electrophoresis

PCR-amplified DNA samples in a 2 % (w/v) agarose gel, prepared using 1× TAE buffer followed by running agarose gel electrophoresis for analyzing cDNA samples. In order to prepare the gel, 0.6 g agarose power was dissolved in 30 ml of 1× TAE buffer and heated till uniformity was achieved. Agarose gel was poured in a gel casting setup consists of tray and comb and then, allowed to set at room temperature after cooling treatment at 50 to 60°C. A suitable nucleic acid dye was then added. Wells were loaded with DNA samples and standard molecular weight marker and 6× loading dye. Until sufficient separation was obtained, electrophoresis was performed in a horizontal unit using 1× TAE buffer at 80-120 V. Using a gel documentation system, the resolved DNA bands were visible under UV light and recorded for analysis. (Sambrook & Russell 2001; Green & Sambrook 2012).

RESULTS

Culture Growth and Biomass Preparation *Armillaria mellea*

Uniform and consistent mycelial growth of *Armillaria mellea* was observed on Malt Extract Agar Media under standardisation. Incubation was done in the dark at 24 ºC. Selected growth parameters allowed for steady, consistent, and healthy colony development. The selected growth parameters supported steady radial expansion and healthy colony morphology, which is characteristic of basidiomycetous fungi. Over prolonged incubation (up to 90 days), the colonies exhibited dense mycelial mats with concentric zonation and pigmentation changes. Distinct rhizomorphic root-like structures were observed extending from the central inoculum plug toward the periphery of the plate. These rhizomorphs appeared as dark, cord-like aggregations of hyphae, a diagnostic morphological feature of *Armillaria* species. Pigmented exudates ranging from reddish-brown to dark black droplets were also released on the colony surface during later stages of growth. For biomass production, the fungus was propagated in Malt Extract Broth (MEB) under similar incubation conditions. Substantial mycelial biomass was obtained in liquid culture, forming compact floating mats and aggregated hyphal masses. The harvested biomass was sufficient for downstream genomic DNA extraction and molecular analysis.

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

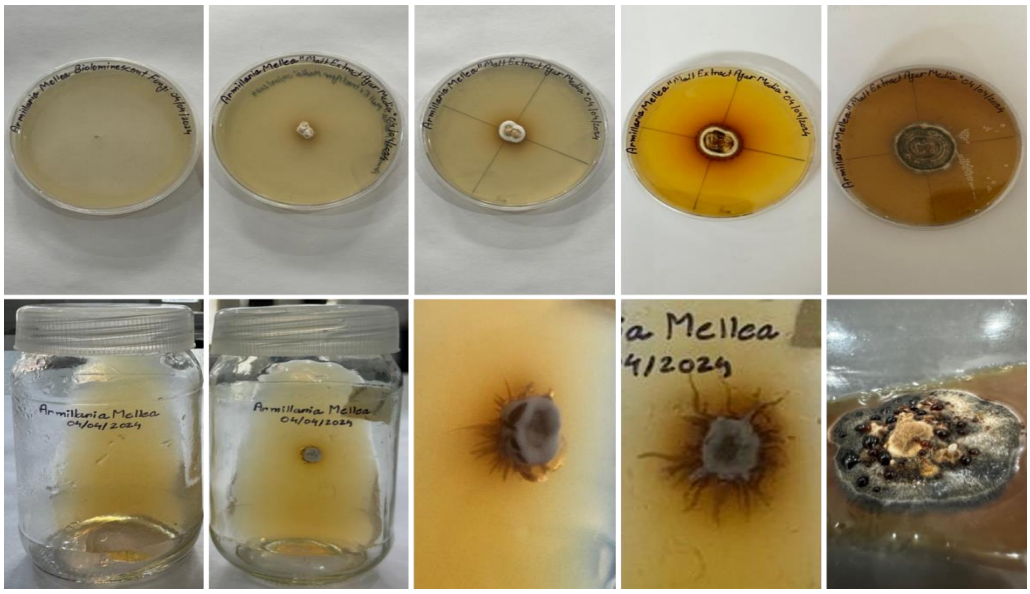


Figure 1: Time-dependent colony development of *Armillaria mellea* on Malt Extract Agar

Progressive radial mycelial growth of *Armillaria mellea* incubated at 24 ^{o}C under dark conditions, showing concentric zonation, rhizomorphic (root-like) structures, and pigmented exudate production in mature (Day 90) colonies (Figure 1).

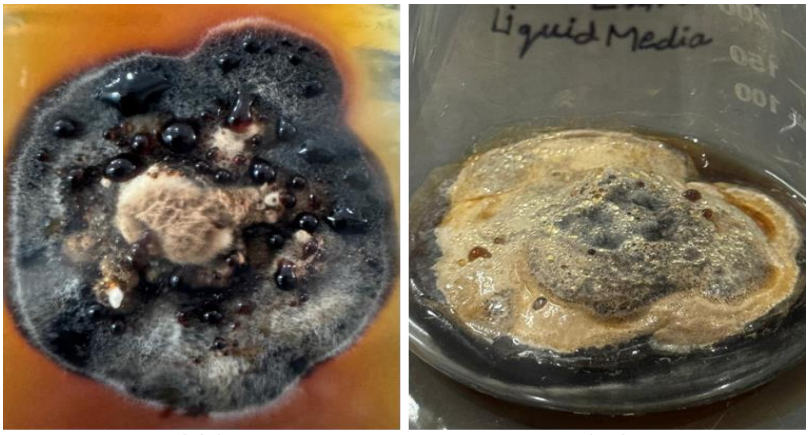


Figure 2: Growth of *Armillaria mellea* in solid and liquid media: (A) Colony morphology on Malt Extract Agar after 90 days of incubation at 24 ^{o}C showing dense mycelial growth and rhizomorphic structures, and (B) Mycelial biomass formation in Malt Extract Broth exhibiting biomass formation in Malt Extract Broth exhibiting compact hyphal aggregation

This is a characteristic feature of basidiomycete fungi and is also applicable for the maintenance of cultures on a routine basis. For propagation, liquid Malt Extract Broth Media was used, and dense mycelial biomass was obtained, which was enough for the extraction of genomic DNA. After 90 days, (A) the adequate development of the *Armillaria mellea* fungus is noticeable in the MEA media, and (B) Adequate development of the *Armillaria mellea* fungus is also noticeable in the broth media. *Armillaria mellea* fungus development was optimal in both solid and liquid media. The mycelium of *Armillaria* releases liquid droplets of different shades, which range from red to black.

Primer Design and Gene Identification

Gene-specific primers amplifying the fungal bioluminescence gene luciferase (Luz) gene of *Armillaria mellea* were custom-synthesized by Barcode Biosciences (India). The LUZ Fwd primer is 21 bp long, while LUZ Rev

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

is 23 bp. The synthesized primers GC (Luz Fwd 55% and Luz Rev 52%) and melting temperatures (Tm) 59.6°C and 60.7°C are within the suitable range 55 to 65°C for the purpose of PCR amplification (Dieffenbach *et al*., 1993; Thornton & Basu 2011). Accurate amplification of the target gene was NCBI Primer-BLAST (Ye et al., 2012) Other than LIG, no secondary structures were reported by OligoAnalyzer (Owczarzy et al., 2008). The synthesis report for the primers documented the Optical Density (OD) value, and Molecular Weight (MW). The primers were used in PCR-based confirmation of the Luz gene (Oba *et al*., 2017; Kotlobay *et al*., 2018).

Primer name	Sequencing (5´ to 3´)	Length (bp)	GC%	Tm (°C)
LUZ Fwd	TGAAACTTGACCTCGTCGGAC	21	52.4	59.8
LUZ Rev	GCAGGGTCATTGGTCATATAGCC	23	52.2	62.4

Table - Luz gene-Specific Primers used in the Study

PCR Validation of the Luz Genes

PCR amplification was performed using gene-specific primers for one of the key enzymes of fungal bioluminescence. The template for amplification was the genomic DNA obtained from the fungal samples. On the agarose gel electrophoresis, a distinct and sharp DNA band of 785 bp was obtained, which corresponds to the expected size of the amplicon for the Luz gene. The specificity and fidelity of the amplification were confirmed due to the absence of nonspecific bands indicating primer-dimer formation. The negative control had no amplification which ruled out contamination. The presence of the Luz gene is strong evidence for the bioluminescent genetic capability of the fungal isolate under study (Oba *et al*., 2017; Kotlobay *et al*., 2018).

Agarose Gel Electrophoresis for Luz Gene Amplification

The Luz gene PCR products were studied using agarose gel electrophoresis. A 2% (w/v) agarose gel was made by mixing ethidium bromide with 30 µL of 1× TAE buffer in 0.6 gram of agarose to visualize the DNA. To determine the size of the band, the gel had 50 base pair (bp) molecular weight markers (ladders) added to the sample wells. The gel was run at 90 to 100 V for 45-60 minutes before being read at the UV gel documentation system. In the experimental well, there was one distinct band, which was 785 bp, matching the expected size of the Luz gene amplicon. There was no other banding with no nonspecific amplifications, which demonstrates the high specificity of the primers with no formation of primer-dimers. No amplification was seen in the negative control to show that there was no contamination. The strong presence of the Luz gene confirms the bioluminescent genetic possibilities of the fungal isolate.

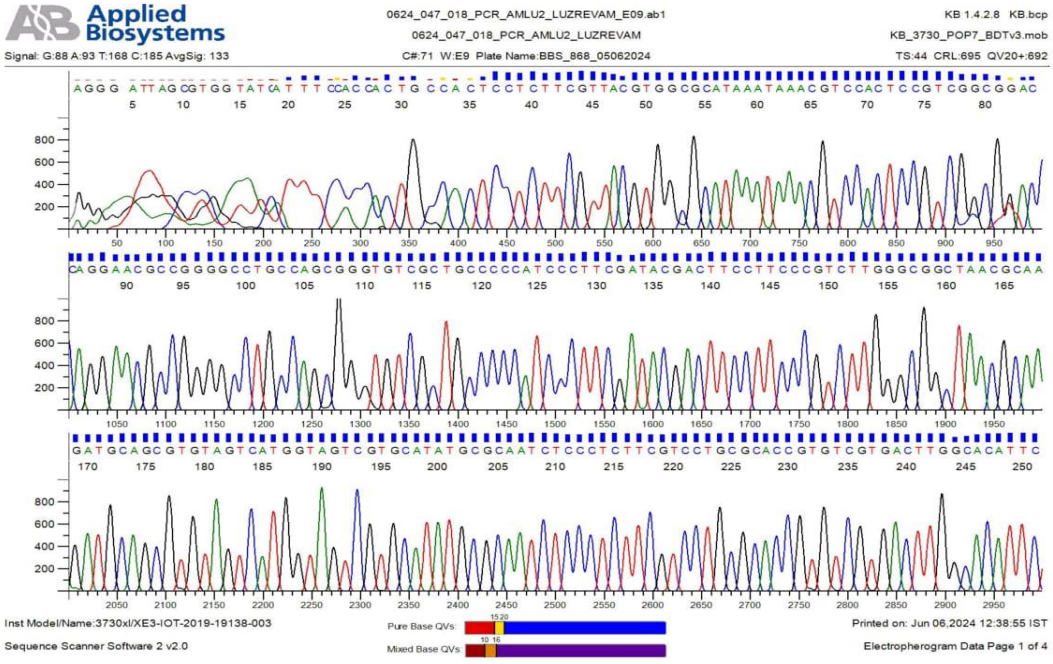
Figure 3: Detection of the Luz gene in a fungal isolate using PCR

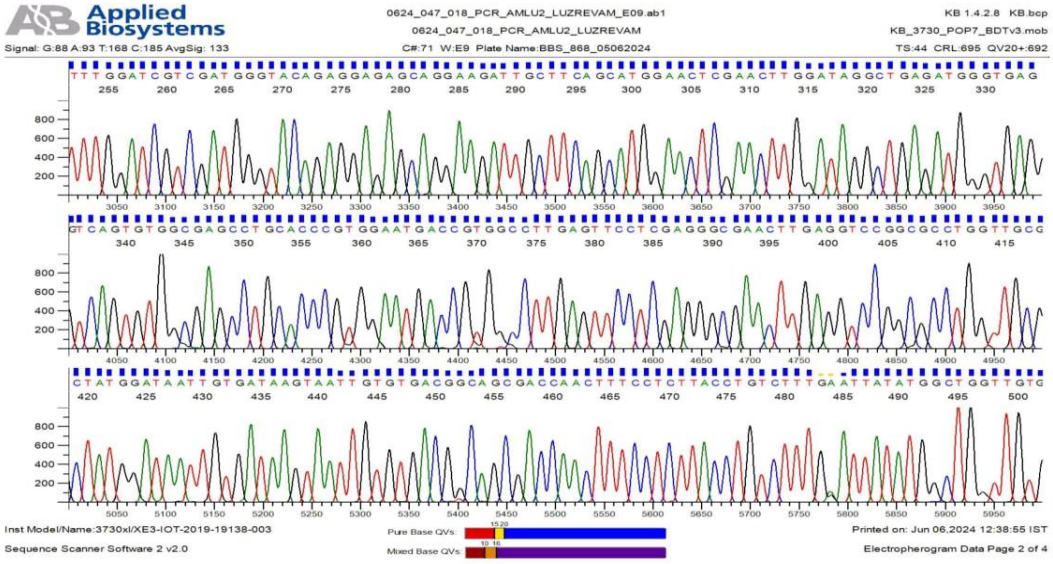
DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

Sanger Sequencing and Gene Confirmation

The purified amplified Luz gene PCR product was analyzed through Sanger Sequencing for molecular verification. The nucleotide sequencing results were compared using the NCBI nucleotide database aligned with the BLASTn tool for checking the base calling. The sequence results matched with the percentage alignment of the Luz gene of Radiant and other similar sequences in the fungal bioluminescence pathway and confirmed that the amplified section pertained to the luciferase gene coding region. The sequencing chromatograms also showed clear peaks and were free of noise, meaning that the sequencing data ware of high quality. The sequenced data ware saved in a FASTA file format for gene annotation and functional analysis, confirming the results.





DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

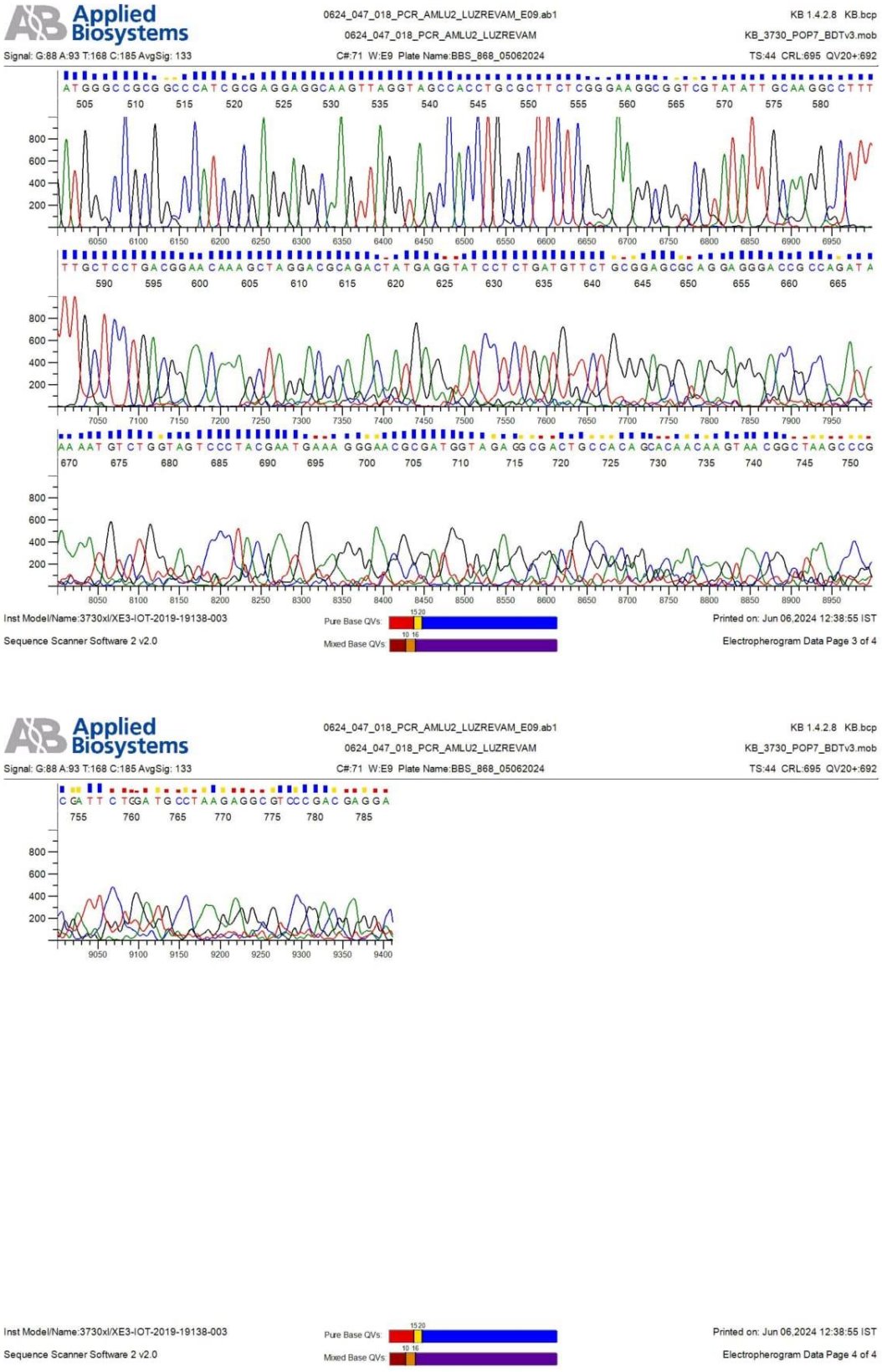


Figure 4: Sanger Sequencing Chromatogram of Luz Gene

Representative Sanger sequencing chromatogram of the amplified Luz gene from *Armillaria mellea*. Clear and well-resolved nucleotide peaks indicate high-quality sequencing data. BLASTn analysis confirmed sequence homology with known fungal luciferase (Luz) gene.

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

Sanger Sequencing and Molecular Confirmation of the Luz Gene

The PCR-amplified fragment of the Luz gene from *Armillaria mellea* was subjected to Sanger sequencing for molecular validation. The obtained nucleotide sequence corresponded to the expected 785 bp amplicon generated using gene-specific primers. Sequence alignment and BLASTn analysis against the NCBI nucleotide database revealed high sequence homology with previously reported fungal luciferase (Luz) gene sequences, thereby confirming the identity and specificity of the amplified product. The sequencing chromatogram exhibited clear and well-resolved peaks, indicating high-quality base calling with minimal background noise. The validated sequence was exported in FASTA format and utilized for downstream annotation, comparative genomic analysis, and functional confirmation of the bioluminescence gene.

>0624_047_018_PCR_AMLU2_LUZREVAM_E09.ab1
AGGGATTAGCGTGGTATCATTTCCACCACTGCCACTCCTCTTCGTTACGTGGCGCATAAATAAA
CGTCCACTCCGTCGGC
GGACCAGGAACGCCGGGGCCTGCCAGCGGGTGTCGCTGCCCCCATCCCTTCGATACGACTTCCT
TCCCGTCTTGGGCGGC
TAACGCAAGATGCAGCGTGTAGTCATGGTAGTCGTGCATATGCGCAATCTCCCTCTTCGTCCTG
CGCACCGTGTCGTGAC
TTGGCACATTCTTTGGATCGTCGATGGGTACAGAGGAGAGCAGGAAGATTGCTTCAGCATGGA
ACTCGAACTTGGATAGG
CTGAGATGGGTGAGGTCAGTGTGGCGAGCCTGCACCCGTGGAATGACCGTGGCCTTGAGTTCC
TCGAGGGCGAACTTGAG
GTCCGGCGCCTGGTTGCGCTATGGATAATTGTGATAAGTAATTGTGTGACGGCAGCGACCAACT
TTCCTCTTACCTGTCT
TTGAATTATATGGCTGGTTGTGATGGGCCGCGGCCCATCGCGAGGAGGCAAGTTAGGTAGCCA
CCTGCGCTTCTCGGGAA
GGCGGTCGTATATTGCAAGGCCTTTTTGCTCCTGACGGAACAAAGCTAGGACGCAGACTATGA
GGTATCCTCTGATGTTC
TGCGGAGCGCAGGAGGGACCGCCAGATAAAAATGTCTGGTAGTCCCTACGAATGAAAGGGAA
CGCGATGGTAGAGGCGAC
TGCCACAGCACAACAAGTAACGGCTAAGCCCGCGATTCTGGATGCCTAAGAGGCGTCCCGACG
AGGA

Figure 5: Raw Nucleotide Sequence of the Amplified Luz Gene from *Armillaria mellea*

Representative nucleotide sequence obtained from Sanger sequencing of the PCR-amplified Luz gene fragment (785 bp) from *Armillaria mellea* BLASTn analysis confirmed high sequence homology with reported fungal luciferase (Luz) gene sequences, validating the identity of the amplified product.

***Armillaria mellea* Data Availability**

The complete genome sequencing data of *Armillaria mellea* strain MTCC isolate 409, have been deposited at the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1401498. This BioProject was submitted by Jayoti Vidyapeeth Women University and represents a mono-isolate genome sequencing project. The assembled genome sequence and associated annotation files are available through the NCBI Genome and Assembly databases linked to this BioProject. The corresponding BioSample is registered under accession number SAMN54565225. The organism is taxonomically classified as Armillaria mellea (NCBI Taxonomy ID: 47429), belonging to the phylum Basidiomycota, class Agaricomycetes. All datasets are publicly available through NCBI and support data reuse, validation, and reproducibility of the genomic analysis.

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

DISCUSSION

The present study provides molecular confirmation of the fungal luciferase (Luz) gene in *Armillaria mellea*, supporting its intrinsic bioluminescent genetic capability. The successful of a 785 bp fragment corresponding to the Luz gene validate the presence of a functional component of the fungal bioluminescence pathway. The luciferase gene has been previously characterized as the central enzyme responsible for light emission in luminous fungi (Oba *et al*., 2017; Kotlobay *et al*., 2018). Uniform mycelial growth observed on Malt Extract Agar and in broth media under controlled conditions confirmed the suitability of the culture system for genomic studies. Basidiomycetes, including *Armillaria mellea*, are known to exhibit active secondary metabolism during vegetative growth, often associated with specialized metabolic pathways such as bioluminescence (Oliveira *et al*., 2015). The characteristic secretion of pigmented droplets further supports active metabolic activity in the culture isolate. PCR amplification using gene-specific primers yielded a single, sharp amplicon of the expected size without nonspecific bands or primer-dimer formation. The absence of amplification in the negative control ruled out contamination, demonstrating the robustness and specificity of the designed primers. Primer design parameters, including GC content and melting temperature, were maintained within optimal ranges to ensure amplification fidelity. Sanger sequencing and BLASTn analysis further confirmed high sequence homology with previously reported fungal luciferase genes involved in the caffeic acid-based bioluminescence pathway (Kotlobay *et al*., 2018). This pathway consists of a minimal multigene cassette including Luz, H3H, CPH, and HispS, collectively responsible for autonomous light production in fungi (Oba *et al*., 2017). Identification of the Luz gene in *Armillaria mellea* aligns with previous genomic studies reporting the conservation of the bioluminescent pathway among luminous basidiomycetes (Oliveira *et al*., 2015). The deposition of genome sequencing data under BioProject accession PRJNA1401498 strengthens the reproducibility and transparency of the study. Public availability of genomic resources facilitates comparative genomics, functional annotation, and exploration of pathway completeness in luminous fungi. Collectively, the molecular evidence presented here establishes a genomic foundation for understanding fungal bioluminescence in *Armillaria mellea*. These finding may contribute to future applications in synthetic biology, autonomous reporter systems, plant transformation studies, and environmentally responsive biosensors, particularly in the context of developing luciferin-independent bioluminescent systems (Kotlobay *et al*., 2018).

CONCLUSION

This study successfully confirms the presence of the fungal luciferase (Luz) gene in *Armillaria mellea* through PCR amplification, agarose gel validation, and Sanger sequencing. The identification of a 785 bp amplicon with high sequence homology to known fungal luciferase genes demonstrates the inherent bioluminescent genetic potential of the isolate. The integration of genomic sequencing, primer validation, molecular amplification, and sequence confirmation provides strong experimental evidence supporting the functional bioluminescence pathway. Public deposition of genomic data further enhances scientific transparency and enables downstream comparative and functional analyses. Overall, this work establishes a molecular framework for future functional characterization of the complete fungal bioluminescence pathway in *Armillaria mellea*, paving the way for potential biotechnological applications involving autonomous light-emitting systems.

DECLARATIONS

Acknowledgment

The authors express their sincere gratitude to the Department/Institutional laboratory facilities for providing the necessary infrastructure and technical support to conduct this research work. The authors gratefully acknowledge Solution Research Laboratory, Cheeka, Haryana, for providing laboratory support and experimental facilities required for fungal culture and molecular work. The authors also acknowledge Anuvanshiki, New Delhi, a genomics services and NGS data analysis solutions provider, for their assistance with genome sequencing, Sanger sequencing, and technical support in sequencing data generation and analysis. The authors further extend their sincere thanks to Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India, for academic support and research guidance.

DOI: 10.22178/acta.26.2.57

ISSN: 2178-7727

Patent: "Not Applicable"

Funding: This research was made possible through financial support in the form of a Junior Research Fellowship (JRF) awarded by the National Testing Agency (NTA).

Institutional Review Board Statement: "Not Applicable"

Informed Consent Statement: "Not Applicable"

Author Contributions

Akriti Singh conceptualization, experimental design, fungal culture maintenance, genomic DNA extraction, primer design, PCR experiments, sequencing validation, data analysis, manuscript drafting, and data submission. Parul Gautam assistance in data interpretation and manuscript editing. Aziz Mohammad Khan general supervision and final review of the manuscript.

Conflict of Interest: "No conflict of interest"

REFERENCE

1. Atlas, R.M., (2010). Handbook of Microbiological Media (4th ed.). CRC Press, Boca Raton, FL. ISBN:9780429130496. DOI: https://doi.org/10.1201/EBK1439804063
2. Bentley, D.R., Balasubramanian, S., Swerdlow, H.P., Smith, G.P., Milton, J., Brown, C.G., Hall, K.P., Evers, D.J., Barnes, C.L., Bignell, H.R., Boutell, J.M., Bryant, J., Carter, R.J., Cheetham, R.K., Cox, A.J., Ellis, D.J., Flatbush, M.R., Gormley, N.A., Humphray, S.J., Irving, L.J., Karbelashvili, M.S., Kirk, S.M., Li, H., Liu, X., Maisinger, K.S., Murray, L.J., Obradovic, B., Ost, T., Parkinson, M.L., Pratt, M.R., Rasolonjatovo, I.M.J., Reed, M.T., Rigatti, R., Rodighiero, C., Ross, M.T., Sabot, A., Sankar, S.V., Scally, A., Schroth, G.P., Smith, M.E., Smith, V.P., Spiridou, A., Torrance, P.E., Tzonev, S.S., Vermaas, E.H., Walter, K., Wu, X., Zhang, L., Alam, M.D., Anastasi, C., Aniebo, I.C., Bailey, D.M.D., Bancarz, I.R., Banerjee, S., Barbour, S.G., Baybayan, P.A., Benoit, V.A., Benson, K.F., Bevis, C., Black, P.J., Boodhun, A., Brennan, J.S., Bridgham, J.A., Brown, R.C., Brown, A.A., Buermann, D.H., Bundu, A.A., Burrows, J.C., Carter, N.P., Castillo, N., Catenazzi, M.C.E., Chang, S., Cooley, R.N., Crake, N.R., Dada, O.O., Diakoumakos, K.D., Dominguez-Fernandez, B., Earnshaw, D.J., Egbujor, U.C., Elmore, D.W., Etchin, S.S., Ewan, M.R., Fedurco, M., Fraser, L.J., Fajardo, K.V.F., Furey, W.S., George, D., Gietzen, K.J., Goddard, C.P., Golda, G.S., Granieri, P.A., Green, D.E., Gustafson, D.L., Hansen, N.F., Harnish, K., Haudenschild, C.D., Heyer, N.I., Hims, M.M., Ho, J.T., Horgan, A.M., Hoschler, K., Hurwitz, S., Ivanov, D.V., Johnson, M.Q., James, T., Jones, T.A.H., Kang, G-D., Kerelska, T.H., Kersey, A.D., Khrebtukova, I., Kindwall, A.P., Kingsbury, Z., Kokko-Gonzales, P.I., Kumar, A., Laurent, M.A., Lawley, C.T., Lee, S.E., Lee, X., Liao, A.K., Loch, J.A., Lok, M., Luo, S., Mammen, R.M., Martin, J.W., McCauley, P.G., McNitt, P., Mehta, P., Moon, K.W., Mullens, J.W., Newington, T., Ning, Z., Ng, B.L., Novo, S.M., O'Neill, M.J., Osborne, M.A., Osnowski, A., Ostadan, O., Paraschos, L.L., Pickering, L., Pike, A.C., Pike, A.C., Pinkard, D.C., Pliskin, D.P., Podhasky, J., Quijano, V.J., Raczy, C., Rae, V.H., Rawlings, S.R., Rodriguez, A.C., Roe, P.M., Rogers, J., Bacigalupo, M.C.R., Romanov, N., Romieu, A., Roth, R.K., Rourke, N.J., Ruediger, S.T., Rusman, E., Sanches-Kuiper, R.M., Schenker, M.R., Seoane, J.M., Shaw, R.J., Shiver, M.K., Short, S.W., Sizto, N.L., Sluis, J.P., Smith, M.A., Sohna, J.E.S., Spence, E.J., Stevens, K., Sutton, N., Szajkowski, L., Tregidgo, C.L., Turcatti , G., Vondele, S.V., Verhovsky, Y., Virk, S.M., Wakelin, S., Walcott, G.C., Wang, J., Worsley, G.J., Yan, J., Yau, L., Zuerlein, M., Rogers, J., Mullikin, J.C., Hurles, M.E., McCooke, N.J., West, J.S., Oaks, F.L., Lundberg, P.L., Klenerman, D., Durbin R., & Smith, A.J. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53-59. DOI: 10.1038/nature07517

ISSN: 2178-7727

3. Dieffenbach, C.W., Lowe, T.M., & Dveksler, G.S. (1993). General concepts for PCR primer design. PCR Methods and Applications. 3(3), S30-S37. DOI: 10:1101/gr.3.3.s30
4. Doyle, J.J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19(1), 11-15. https://webpages.charlotte.edu/~jweller2/pages/BINF8350f2011/BINF8350_Readings/Doyle_plantDNAextractCTAB_1987.pdf
5. Green, M, R., & Sambrook, J. (2012). Molecular Cloning: A Laboratory Manual (4th ed.). Cold Spring Harbor Laboratory Press. ISBN: 978-1-936113-42-2.
6. Kotlobay, A.A., Sarkisyan, K.S., Mokrushina, Y.A., Marcet-Houben, M., Serebrovskaya, E.O., Markina, N.M., Gonzalez Somermeyer, L., Gorokhovatsky, A.Y., Vvedensky, A., Purtov, K.V., Petushkov, V.N., Rodionova, N.S., Chepurnyh, T.V., Fakhranurova, L.I., Guglya, E.B., Ziganshin, R., Tsarkova, A.S., Kaskova, Z.M., Shender, V., Abakumov, M., Abakumova, T.O., Povolotskaya, I.S., Eroshkin, F.M., Zaraisky, A.G., Mishin, A.S., Dolgov, S.V., Mitiouchkina, T.V., Kopantzev, F.P., Waldenmaier, H.E., Oliveira, A.G., Oba, Y., Barsova, E., Bogdanova, E.A.T., Stevani, C.V., Lukyanov, S., Smirnov, I.V., Gitelson, J.I., Kondrashov, F.A., & Yampolsky, I.V. (2018). Genetically encodable bioluminescent system from fungi. Proceedings of the National Academy of Sciences of the United States of America. 115(50), 12728–12732. DOI: https://doi.org/10.1073/pnas.1803615115
7. Oba, Y., Ohmiya, A., & Sakakibara, H. (2017). Identification of the fungal luciferin pathway for autonomous luminescence in plants. Science Advances. 3(1), e1602402. DOI: https://doi.org/10.1126/sciadv.1602402
8. Oliveira, A.G., Stevani, C.V.,Waldenmaier, H.E., Viviani, V., Emerson, J.M., Loros, J.J., & Dunlap, J.C. (2015). Circadian Control Sheds Light on Fungal Bioluminescence. Current Biology. 25(7), 964-968. DOI: https://doi.org/10.1016/j.cub.2015.02.021
9. Owczarzy, R., Tataurov, A.V., Wu, Y., Manthey, J.A., McQuisten, K.A., Almabrazi, H.G., Pedersen, K.F., Lin, Y., Garretson, J., McEntaggart, N.O., Sailor, C.A., Dawson, R.B., & Andrew, S.P. (2008). IDT SciTools: a suite for analysis and design of nucleic acid oligomers. Nucleic Acid Research. 36, W163-W169. DOI: 10.1093/nar/gkn198
10. Sambrook, J., & Russell, D.W. (2001). Molecular Cloning: A Laboratory Manual, 3rd Edition. Cold Spring Harbor Laboratory Press. http://www.cshlpress.org/
11. Sperschneider, J., Gardiner, D.M., Dodds, P.N., Tini, F., Covarelli, L., Singh, K.B., Manners, J.M., & Taylor, J.M. (2015). EffectorP: predicting fungal effector proteins form secretomes using machine learning. New Phytol. 210(2), 743-761. DOI: https://doi.org/10.1111/nph.13794
12. Thornton, B., & Basu C. (2011). 39(2), 145-154. Real-time PCR (qPCR) primer design using free online software. Biochem Mol Biol Educ. 39(2), 145-154. DOI: 10.1002/bmb.20461
13. Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T.L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 13, 134. DOI: 10.1186/1471-2105-13-134