

Research article**LC-MS Analysis and Intracellular and Extracellular Indole 3 Acetic Acid Production under Different Media by Endophytic Bacteria Associated with *Humulus lupulus*****Sohail Khan and Ashwani Mathur****Department of Biotechnology, Jaypee Institute of Information Technology, Noida A-10, Sector-62, Noida-201309, Uttar Pradesh, India*

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Abstract

Recently, there has been a worldwide call to explore nature-friendly metabolites, which could enhance plant growth and substitute for chemically synthesized products. Indole-3-acetic acid (IAA) is one of the versatile metabolites that has a potential role for plant growth, anti-inflammatory, hepatoprotective, and anticancer properties. Furthermore, IAA is commonly synthesized chemically; the majority of reagents used pose environmental pollution. In contrast, biosynthesis through controlled cultivation of endophytes from medicinal plants offers an environmentally sustainable approach. The current study investigates the endophytic bacterium *Bacillus licheniformis* SKAM1 isolated from the leaves of *Humulus lupulus* for IAA production. The identification and characterization of endophytic bacterium was carried out using biochemical and molecular methods. Furthermore, LC-MS analysis of the dried extract of *Bacillus licheniformis* SKAM1 identified multiple bioactive compounds, including IAA, with potential therapeutic and agricultural applications. Additionally, the IAA quantification was performed using ultra-performance liquid chromatography (UPLC) across different media. UPLC analysis reveals that *Bacillus licheniformis* SKAM1 produces IAA in Luria broth medium; the extracellular IAA concentration was determined to be 1.16 mg/mL, whereas the intracellular level reached 1.11 mg/mL. Similarly, culture in minimal medium with extracellular IAA produced at 0.11 mg/mL and intracellular IAA at 0.06 mg/mL. The current study paves the way for exploring the role of abiotic conditions for cost-effective IAA production.

Keywords: endophytes, metabolite, bacterial sustainability, Indole-3-acetic acid, medicinal plant

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1. Introduction

The isolation and characterization of microorganisms from diverse habitats have been pivotal to exploring their genetic diversity and metabolite fingerprints (Khan et al., 2025). The 'endophytes', as the term was introduced by de Barry in 1866 (Collinge et al., 2022), are the plant-colonizing non-pathogenic microorganisms that colonize different tissues of the plants and asymptotically assist in plant growth and metabolism, inhabiting plant tissues, either for a portion of their lifespan or for their entire lifespan (Malinowski & Belesky, 2000; Haggag, 2010; Basumatary et al., 2021; Wu et al., 2021). The growing scientific interest in the exploration of endophytes from plants is due to their role in plant protection, growth enhancement, and assisting plant metabolism (Wei et al., 2020; Emtaro et al., 2024). When produced through environmentally friendly technology, these products can offer significant advantages for both farmers and users. The demonstration of plant endophytic bacteria showed host-specific characteristics and could be influenced by environmental circumstances (Zou et al., 2023a). Extensive investigations have reported the modulatory effect of endophytes on enhancing plant growth and safeguarding plants from pathogens. The endophytes are attracting scientific attention as a source of novel natural products because of their crucial relationship with plants (Singh et al., 2017). Plant metabolites provide a rich source of pharmacologically significant compounds that promote drug discovery (Atanasov et al., 2015). However, the classical technique of harvesting secondary metabolites from medicinal plants encounters several obstacles, such as seasonal variability, mismatches between market needs and availability, erosion of biodiversity, the threatened status of various plant species, and rising costs (Patakova et al., 2024). It should be noted that plants are not always the exclusive producers of their metabolites. Often, the association of endophytes and the biosynthesis of metabolites regulate biosynthesis (Mishra et al., 2022). One of such plant growth metabolites is IAA. IAA is a versatile molecule that plays roles in plant growth regulation, tissue differentiation, cell elongation, proliferation, and specialization in response to environmental signals (Teale et al., 2006). Furthermore, earlier investigations have accentuated the possible therapeutic role of IAA, particularly its anti-inflammatory effects (Shen et al., 2022), hepatoprotective activity (Stofan & Guo, 2020), anticancer properties (Folkes & Wardman, 2001), and its capacity to potentiate the effectiveness of chemotherapy in pancreatic cancer treatment (Seo & Wargo, 2023). In addition, the worldwide demand for IAA is rising, and the market is forecasted to attain USD 36 million by 2028 (Arora et al., 2024). While IAA is traditionally synthesized through chemical processes, the majority of chemicals utilized in these processes are associated with environmental hazards. In contrast, the biosynthesis of IAA via artificial cultivation of endophytes isolated from natural habitats, such as medicinal plants, offers a greener and more sustainable strategy (Ham et al., 2021). Previous studies have shown that endophytes isolated from medicinal plants such as *Camellia sinensis*, *Moringa peregrina*, *Kalanchoe pinnata*, and *Humulus lupulus* produce IAA (Khan et al., 2016; Hazarika et al., 2021; Renugadevi et al., 2022; Khan & Mathur, 2025; Patakova et al., 2024). Furthermore, *Humulus lupulus* L. (hop) is a dioecious, perennial, herbaceous climber classified within the family Cannabaceae. The species is largely native to the temperate regions but is also universally cultivated because of its production of secondary metabolites. These metabolites are responsible for the characteristic bitterness, flavor, aroma, and antimicrobial properties of beer (Zanoli & Zavatti, 2008). In addition to their role in brewing, several hop-derived compounds possess bioactive properties with significant pharmaceutical relevance, particularly as sedative and antimicrobial agents (Lamy et al., 2007; Liu et al., 2015). Research on *Humulus lupulus* has, until now, primarily explored

areas unrelated to its colonization by endophytic and epiphytic bacteria, and only a small number of studies have considered this aspect (Altschul et al., 1990; Goryluk-Salmonowicz et al., 2016; Allen et al., 2019; Micci et al., 2022). On the other hand, researchers widely considered that hop plants would not harbor bacterial colonizers, given that a substantial proportion of their metabolites possess antimicrobial properties (Bocquet et al., 2018). Although bacterial diseases of hops are observed far less often than viral and fungal diseases, several bacterial taxa have been identified. These include *Streptomyces* spp. from the rhizosphere (Kolek et al., 2021), *Pseudomonas stutzeri* and *Pseudomonas fluorescens* from cones, and *Pantoea agglomerans* from cones (Sevigny et al., 2019), as well as dried hop pellets (Kolek et al., 2021). Previous studies have reported that plant-associated bacterial endophytes commonly belong to the genera *Pseudomonas*, *Bacillus*, *Pantoea*, and *Streptomyces* (Shurigin et al., 2022; Tao et al., 2022).

The current study highlighted the isolation and characterization of an endophytic bacterium from *Humulus lupulus*. The dried extract of an isolated endophytic bacterium was analyzed using LC-MS, revealing multiple metabolites with potential applications in both therapeutic and agricultural fields. In addition, ultra-performance liquid chromatography (UPLC) was employed for the quantitative analysis of metabolites, with particular focus on IAA. The findings reveal that the endophytic bacterium was capable of producing IAA, underscoring its potential for biotechnological application.

2. Materials and Methods

2.1 Plant material and bacterial isolation

Fresh and healthy plant leave was collected from *Humulus lupulus* and maintained at the campus premises. The endophytic bacterium was isolated as described previously by Sharma et al. (2021). Briefly, the collected leaves were initially cleaned with tap water to eliminate surface debris, followed by two rinses with distilled water. Subsequently, the plant materials were surface sterilized in 70% ethanol for 1 min, treated with 4% sodium hypochlorite solution for 5 min, and washed thrice with autoclaved distilled water within the laminar hood. The leaf was excised and placed on Luria agar plates. The plates were incubated at 37°C for 24 h. The growth around the excised leaves was monitored and transferred to sterile Luria agar plates using a sterile loop. The plates were incubated at 37°C for 24 h for growth and then stored at 4°C for further use.

2.2 Morphological characteristics and molecular identification of endophytes

The endophytic bacteria were morphologically and biochemically characterized using Gram staining and a HiBacillus identification kit (KB013) from Himedia Laboratories Pvt. Ltd, Mumbai, India. This kit consists of 12 biochemical tests: trehalose, Voges-Proskauer, ONPG, citrate, catalase, arginine, sucrose, arabinose, nitrate reduction, mannitol, glucose, and malonate (Venkataramanamma et al., 2022). Molecular identification of an isolated endophytic bacterium was performed using the molecular technique of the 16S rRNA gene sequencing, as reported previously by Tao et al. (2022) with modifications. The primer sequences for the forward (27F) and reverse (1492R) were procured from Barcode Bioscience, India, and were used (Table 1). The PCR reaction mixtures were prepared with a total volume of 30 µL containing 3 µL 2mM dNTPs, 1.5 µL of Taq Polymerase 10mM, 3 µL of 10x Buffer, 1.5 µL of both primers (20 pmol), and deionized water. A cream-whitish-colored colony was chosen as the DNA template for PCR. The colony was picked with

sterile toothpicks and suspended in 500 μ L autoclaved water. The amplification methods involved a series of temperature changes through thermal cycling parameters at 94°C for 5 min, 35 cycles for denaturation at 94°C for 30 s, and a synthesis procedure at 72°C for 90 s. and then a final extension at 72°C for 5 min. Deionized water was used as a negative control to monitor contamination. The amplified product was subjected to gel electrophoresis on 0.8% agarose gel to visualize and confirm its size. Sanger sequencing was performed using the Seq Studio Flex Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA sequence was aligned using the Basic Local Alignment Search Tool (BLAST). A phylogenetic tree was constructed using the neighbor-joining method in MEGA 11.0 (Molecular Evolutionary Genetics Analysis software) with multiple sequence alignment performed using CLUSTALW (Medison et al., 2023; Kholikov et al., 2025). To assess reliability, a bootstrap analysis was conducted with 1000 replications.

Table 1. Details of the forward and reverse primers used for 16S rRNA gene amplification

S. No	Primer Name	Primer Length	Sequence	GC Content	Melting temperature (as per manufacturer's specification)
1	Forward Primer (27F)	22	5-AGAGTTTGATCMTGGCTCAG-3	47.5	56.3°C
2	Reverse Primer (1492R)	19	5-GGTTACCTTGTTACGACTT-3	42.1	52.4°C

2.3 Culture conditions and media comparison for IAA production

To assess extracellular production of IAA, the endophytic bacterium *Bacillus licheniformis* SKAM1 was cultivated in Luria broth media (HiMedia, Mumbai, India) and M9 Minimal Medium Salts (1X) (HiMedia, Mumbai, India). The bacterial cells were harvested from the broth via centrifugation. The supernatant was dried at room temperature. To assess intracellular production of IAA from the endophytic bacterium *Bacillus licheniformis* SKAM1, the method adopted by Singh et al. (2023) was followed. The bacterial cell pellet was resuspended in 1 mL of methanol (Sigma, HPLC grade). The resulting mixture was subjected to sonicated at 80 A° for 2 min with 10 s on-and-off cycles. Subsequently, the sample was centrifuged at 10,000 RPM for 10 min at 4°C to obtain the supernatant containing the sample mixture, which was then aliquoted and stored at -80°C until further analysis.

2.4 LC-MS analysis of dried extract

To detect the IAA, the dried extract of the endophytic bacterium was analyzed by LC-MS, and it was carried out as described by Kuźniar et al. (2021) with modification. The Dionex Ultimate 3000 (Thermo Scientific) fitted with a Hypersil Gold C18 column (2.1mm x 100mm, 3.0 μ m) at a temperature of 25°C was used. The flow rate employed was at 0.320 mL/min (320 μ L/min). The method duration was 8 min. The mobile phase consisted of buffer A: 0.1% formic acid in water and buffer B: 0.1% formic acid in methanol. Q Exactive, Thermo Scientific was used for mass spectrometry (MS) detection, and ESI mode was used for the ionization with probe heater temp: 320°C, capillary temperature: 270°C, capillary voltage: (+) 4.0 kV, aux gas flow rate (arbitrary unit): 5, S-Lens RF Level: 50, sheath gas flow rate

(arbitrary unit): 30, sweep gas flow rate: 0, scan range: 100-1500 m/z, resolution: 70,000. Thermo Fisher Scientific Compound Discoverer 3.3 was used for the identification and investigation of the bioactive compounds

2.5 Thin-layer chromatography of IAA

TLC was conducted by spotting the standard IAA and sample onto TLC silica gel (60GF254, 20 × 20 cm, Merck). A mobile phase consists of ethyl acetate: chloroform: formic acid (55:35:10, v/v/v). Spots exhibiting R_f values identical to authentic IAA were identified under UV light with a wavelength of 254 nm (Mohite, 2013).

2.6 Determination of IAA by UPLC

Quantification of IAA was carried out as described by Szkop and Bielawski (2013). UPLC analysis of the IAA from the endophytic bacterium *Bacillus licheniformis* SKAM1 was conducted using a C18 column. The sample was dissolved in 1 mL of HPLC-grade methanol and exposed to chromatographic separation at room temperature. In this procedure, a Waters Acquity UPLC system was used with BEH C18 column (100mm×2.1mm), mobile phase of solvent A: B (40: 60) (0.01% glacial acetic acid in water: acetonitrile), wavelength: 265nm, column temp.: 35, particle size 1,7um, detector used: PDA eλ detector and run time: 5 min. The flow rate of the mobile phase was kept at 0.3 mL/min, with an injection volume of 1 µL of the respective sample. The generated peak was compared with the retention time of authentic IAA. Indole-3-acetic acid (Central Drug House (P) Ltd), solubilized in HPLC-grade methanol, was used as the standard.

2.7 Statistical analysis

In this investigation, experiments were conducted twice, and the obtained outcome illustrates the average of the observations.

3. Results and Discussion

3.1 Bacterial isolation and identification of endophytic bacteria

Medicinal plants are extensively documented as reservoirs of bioactive compounds, numerous ones of which find application in both traditional and modern medicine. Recently, heightened attention has been directed toward their associated endophytic bacteria, which not only support plant health but also emerge as valuable sources of pharmacologically active metabolites (Alvin et al., 2014). The metabolite-assisted benefits, conferred by endophytes, especially bacterial endophytes, have paved the way for the exploration of less or unexplored habitats and comparing the impact of the microenvironment in modulating metabolite fingerprints. The current study explored an endophytic bacterium isolated from the medicinal plant *Humulus lupulus*. The isolated endophyte bacterium underwent morphological characterization, Gram staining, and biochemical tests. It was identified as *Bacillus* sp. Subsequent molecular identification involved polymerase chain reaction (PCR) amplification of the 16S rDNA gene from genomic DNA, followed by sequencing, which confirmed the bacterium as *Bacillus licheniformis* (Accession No PX363043.1). Homology analysis using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) indicated approximately 100% similarity with *Bacillus licheniformis*. A phylogenetic tree was

constructed using the neighbor-joining method based on the obtained sequence, further validating the species-level identification as *Bacillus licheniformis* SKAM1. Figure 1 shows the phylogenetic tree of the isolated endophyte bacterium. A comprehensive literature review revealed that *Bacillus licheniformis* has not been previously reported from *Humulus lupulus*; thus, this study documents its first isolation from this host plant. *Bacillus licheniformis*, a Gram-positive bacterium capable of forming spores, is highly valued in biotechnology due to its diverse applications. It is utilized in various industries, including aquaculture, agriculture, food production, biomedicine, and pharmaceuticals, for the synthesis of bioactive compounds (Muras et al., 2021). *Humulus lupulus* is widely renowned not only for its economic value in brewing but also as a medicinal plant enriched with prenylated flavonoids, xanthohumol, and bitter acids, with well-documented antimicrobial, anticancer, and antioxidant properties (Riccioni et al., 2025). Although several studies have been conducted on the diversity and functional potential of endophytic fungi from *Humulus lupulus* (Riccioni et al., 2025), information on its bacterial endophytes remains scarce. Recent research has been focused on fungal isolates with antagonistic activity against phytopathogens, whereas bacterial communities have been only scarcely explored (Krofta et al., 2021). Furthermore, there is a critical lack of knowledge about bacterial endophytes that contribute towards metabolite synthesis, plant growth, and stress tolerance. In addition, the ability of *Humulus lupulus*-associated endophytic bacteria to produce bioactive compounds has received limited investigation when compared with endophytes from other medicinal plants (Strobel & Daisy, 2003; Santoyo et al., 2016; Mametja et al., 2025).

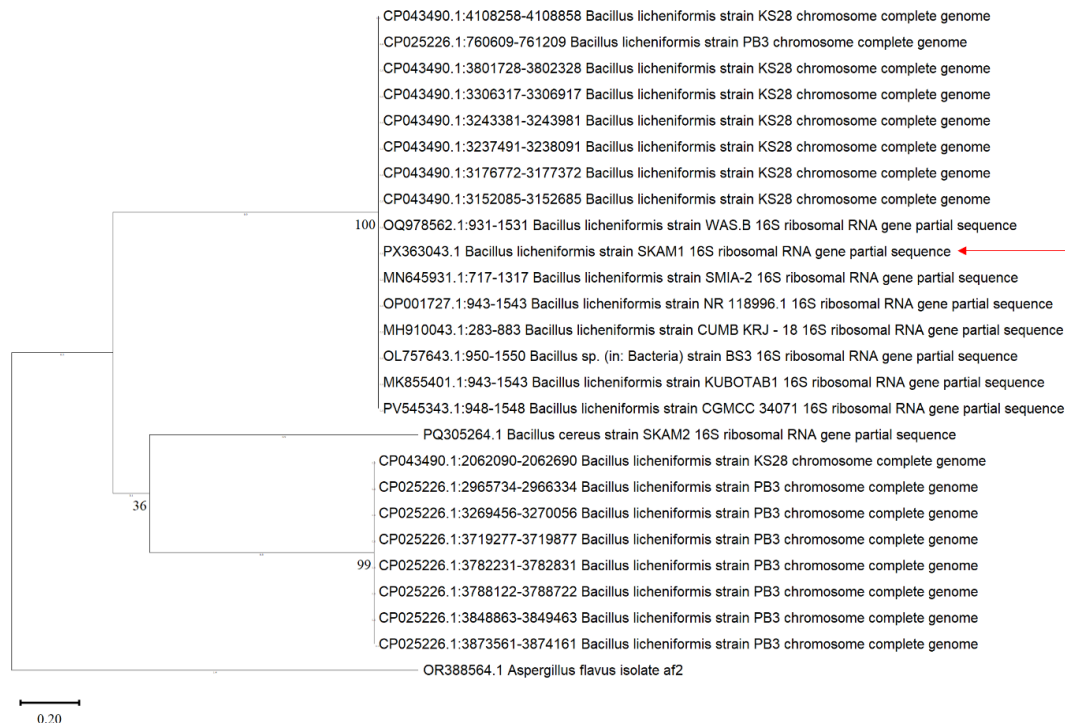


Figure 1. Phylogenetic tree of the isolated bacterium *Bacillus licheniformis* SKAM1

3.2 LC-MS analysis of dried extract

The LC-MS analysis of the dried extract from the endophytic bacterium revealed the presence of several metabolites, many of which are reported to have bioactive and agricultural potential. The chromatographic separation showed distinct peaks at different retention times, indicating a diverse metabolite profile. Table 2 describes the molecular formula, retention time, and PubChem ID of detected compounds and their application. In addition, Figure 2 shows the chromatogram of the LC-MS of the dried extract. A diverse range of metabolites was revealed from the LC-MS analysis, including IAA and other compounds. Among agriculturally and therapeutically important compounds, IAA was highlighted for its pivotal role in promoting plant growth (Arora et al., 2024). Furthermore, compounds with carbofuran activity were identified as widely associated with pesticidal properties (Liang et al., 2023). L-isoleucine, which was also detected, is known to enhance plant resistance mechanisms (Li et al., 2021). The rise of antimicrobial resistance in bacterial pathogens is increasingly posing a serious threat to global public health (Jeong et al., 2022, 2023). The bioactivities of therapeutic compounds, such as their antibacterial, antifungal, and antiviral properties, exhibit versatile roles in combating infectious diseases. Quinolone compounds were documented for their applications in antimicrobial drug development (Drica et al., 2009). Additionally, tryptophol demonstrated antifungal properties (Kitisin et al., 2023), while picolinic acid exhibited antiviral activity (Narayan et al., 2022). The role of endophyte bacterial metabolites, as therapeutic and commercial natural metabolites, has gained significant relevance in the last few decades (Gouda et al., 2016). However, the uniqueness of some of the proposed metabolites was detected using LC-MS analysis further obtrudes on the quest for their characterization and purification. This work may be augmented to include the purification of bioactive compounds and an assessment of their bioactivities.

3.3 Qualitative assessment of IAA by thin-layer chromatography

The qualitative assessment of IAA produced by the endophytic bacterium *Bacillus licheniformis* SKAM1 was validated via thin-layer chromatography (TLC) analysis. The banding pattern monitored for the purified IAA closely resembled that of the standard IAA, indicating a similar composition. The retention factor (R_f) value was calculated to be 0.99. Figure 3 shows the TLC of IAA from the dried extract of the endophytic bacterium *Bacillus licheniformis* SKAM1. Comparable strategies were reported in previous studies where TLC was successfully utilized to confirm the IAA production by endophytic bacteria, with R_f values highly consistent with those of standard IAA (Renugadevi et al., 2022; Khianngam et al., 2023; Zou et al., 2023b). Therefore, while the TLC result delivers qualitative evidence of IAA biosynthesis, supplementary techniques such as HPLC are essential for accurate quantification (Szkop & Bielawski, 2013).

3.4 Quantification of IAA using UPLC

The amount of IAA from a dried extract of *Bacillus licheniformis* SKAM1 was further quantified via UPLC. IAA quantification was conducted using a standard graph method. To quantify IAA, a calibration curve was constructed using five different concentrations of the IAA standard solution. Figure 4 presents the overlay of chromatographic profiles

Table 2. Characteristic properties of the compounds found in dried extract LC-MS analysis

S. No	Identified Compound	PubChem ID	RT	Relative Abundance (%)	mzCloud Best Match Confidence	Molecular Formula	Activity	References
1	L-Phenylalanine	6140	1.48	12.87%	9.5	C9 H11 N O2	Synthesis of neurotransmitters	(Gammoh et al., 2024)
2	(R,S)-Anatabine	261474	1.896	6.92%	9.2	C10 H12 N2	Treatment of Alzheimer's disease Treatment of multiple sclerosis anti-inflammatory Antiparasitic	(Messinis et al., 2022) (Weber et al., 2019)
3	D-(+)-Tryptophan	9060	2.029	6.37%	50.1	C11 H12 N2 O2	Precursor of IAA	(Wary et al., 2022)
4	Indole-3-acetic acid	802	4.745	0.071%	9.6	C10 H9 N O2	Plant growth-promoting	(Arora et al., 2024)
5	Carbofuran	2566	4.647	1.13%	-	C12 H15 N O3	pesticides	(Liang et al., 2023)
6	Picolinic acid	1018	1.095	3.15%	64.6	C6 H5 N O2	Antiviral	(Narayan et al., 2022)
7	L-Isoleucine	6306	1.211	1.22%	9.7	C6 H13 N O2	Plant Resistance	(Li et al., 2021)
8	Tryptophol	10685	4.744	0.72%	-	C10H11NO	Antifungal	(Kitisin et al., 2023)
9	quinolone	6038	2.028	0.35%	-	C9H7NO	Antimicrobial drug	(Drlica et al., 2009)

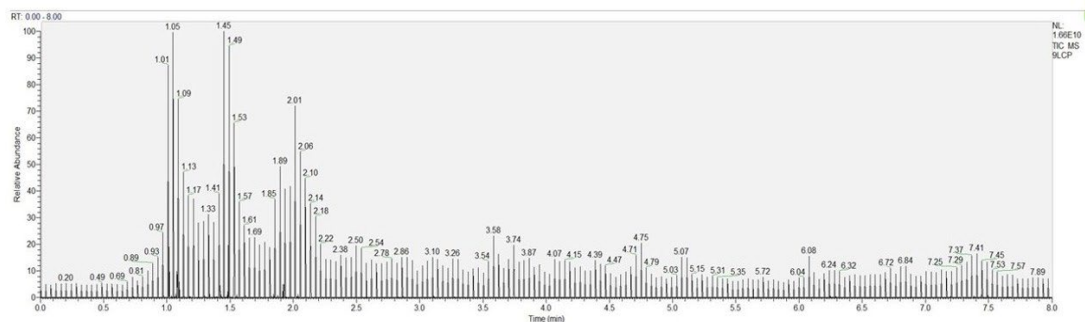


Figure 2. Chromatogram of LC-MS analysis of dried extract of isolated endophyte *Bacillus licheniformis* SKAM1

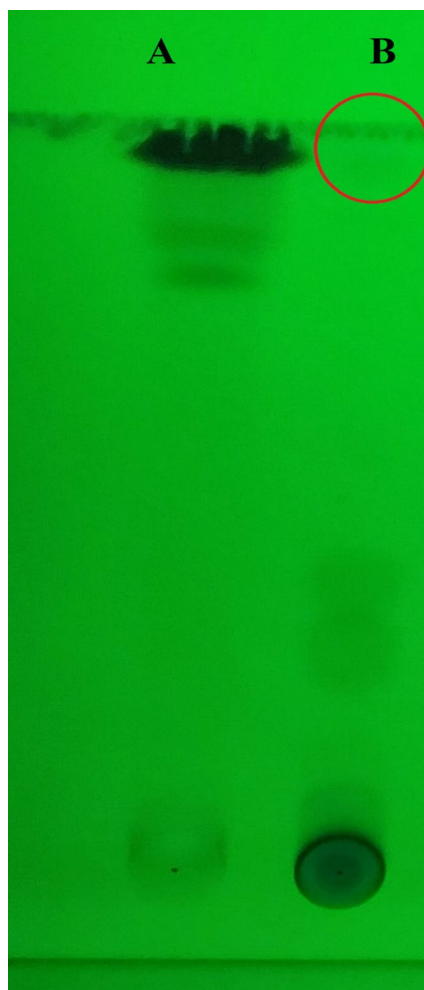


Figure 3. TLC of IAA, banding pattern A: shows the standard of IAA, banding pattern B: shows the IAA from the dried extract.

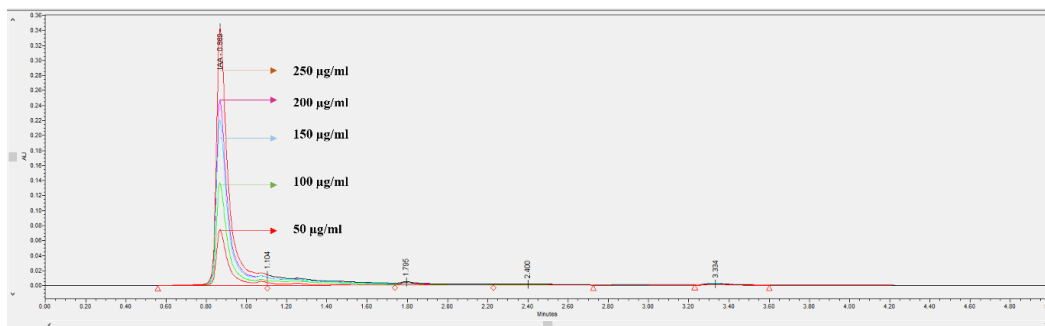


Figure 4. Representative UPLC chromatogram of IAA standard profile

corresponding to these five concentrations. The dried extract of *Bacillus licheniformis* SKAM1, cultured from Luria media, was found to contain IAA at 1.16 mg/mL extracellularly and 1.11 mg/mL intracellularly. Luria broth is extensively employed in microbial metabolite studies because of its complex and nutrient-rich composition (Demain & Fang, 2000; Sezonov et al., 2007). Previous studies explored the IAA production from the bacteria in Luria broth (Luziatelli et al., 2021). Figures 5 shows the UPLC chromatograms of the produced IAA in Luria broth. Furthermore, IAA quantification was done in minimal media, and the amount of IAA was found extracellular at 0.11 mg/mL and 0.06 mg/mL intracellular. Figure 6 presents the UPLC chromatograms of the produced IAA in minimal media (1x), from *Bacillus licheniformis* strain. Minimal media is extensively employed in microbial research as it delivers a chemically defined growth environment. In contrast, complex media like Luria broth contain undefined nutrient compositions (Kim & Kim, 2017; Sigurdarson et al., 2020). Minimal media are considered economical alternatives and appealing substrates for industrial fermentation processes (Rugbjerg et al., 2018). The current result suggests a further optimization study to explore the minimal media for IAA production. IAA is an auxin that governs various aspects of plant growth. It is water-soluble and can passively diffuse across cell membranes when protonated, without requiring a specific transporter (Spaepen et al., 2007; Lu et al., 2018). The ability to produce IAA is regarded as a valuable criterion for screening beneficial microorganisms. Additionally, plants utilize the production of phytohormones, including IAA, to support their immediate growth and to mitigate both biotic and abiotic stresses (Duca et al., 2014; Kim et al., 2017; Shilev et al., 2020). Moreover, bacteria utilize their capability to synthesize IAA as a means to engage with plants. The IAA produced by bacteria plays a crucial role in enhancing root development in plants, consequently improving their ability to absorb water and minerals from the soil (Kang et al., 2021; Khan et al., 2021). To generate non-toxic agricultural products and minimize the reliance on chemical herbicides, potential IAA-producing microbes present a promising option for the production of IAA. Urgent development of biological resources for nature-friendly farming is necessary to mitigate ecosystem pollution resulting from the current overuse of toxic agricultural chemicals (Bunsangiam et al., 2021). Furthermore, *Bacillus* species are extensively studied as plant growth-promoting bacteria that often produce IAA. Previous studies explored *Bacillus* spp. as significant producers of IAA and emphasize their pivotal role in microbe–plant communication (de O. Nunes et al., 2023). Specifically, *Bacillus licheniformis* was commonly reported to produce significant quantities of IAA and to promote plant growth under both laboratory and greenhouse conditions. Previous studies have further explored the IAA biosynthesis by *Bacillus licheniformis* isolates obtained from endophytic environments (de O. Nunes et al.,

2023). Moreover, prior investigation has shown that IAA was produced from endophytic bacteria, *Bacillus cereus* HRT1 (7.8 ± 0.2 $\mu\text{g/mL}$), *Bacillus aryabhatai* HSN1 (8.9 ± 0.3 $\mu\text{g/mL}$), and *Bacillus megaterium* HST16 (8.4 ± 0.3 $\mu\text{g/mL}$) (Shurigin et al., 2022). A previous study also quantified the IAA from endophyte bacteria *Bacillus megaterium* (1.346 ± 0.103 $\mu\text{g/mL}$) isolated from *Paris polyphylla* var. *yunnanensis* (Paris L.) (Tao et al., 2022). Furthermore, IAA production by endophytic bacteria is commonly observed in the extracellular fraction. However, our results indicate that strain *Bacillus licheniformis* SKAM1 produces IAA both intracellularly and extracellularly, contributing to a higher cumulative yield of the IAA.

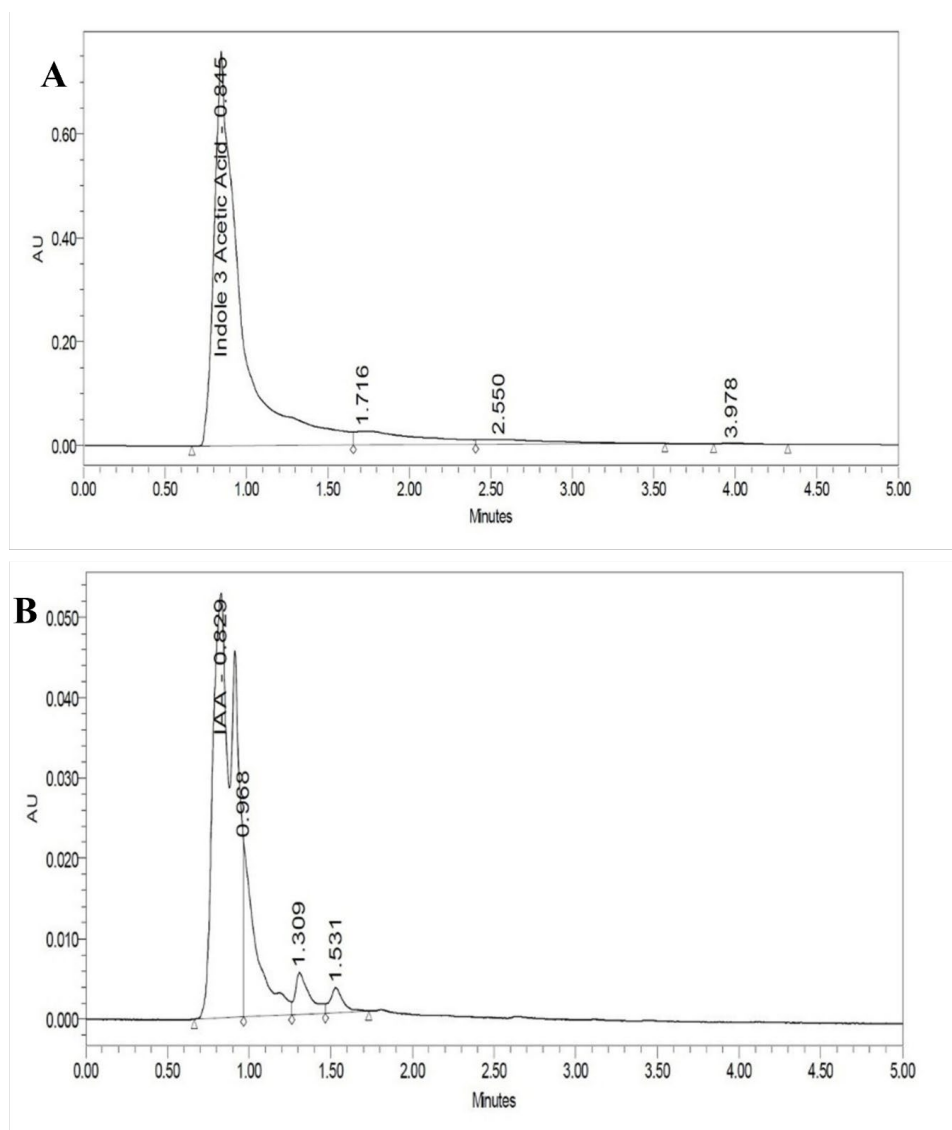


Figure 5. UPLC chromatogram of extracellular (A) and intracellular (B) IAA production in LB media

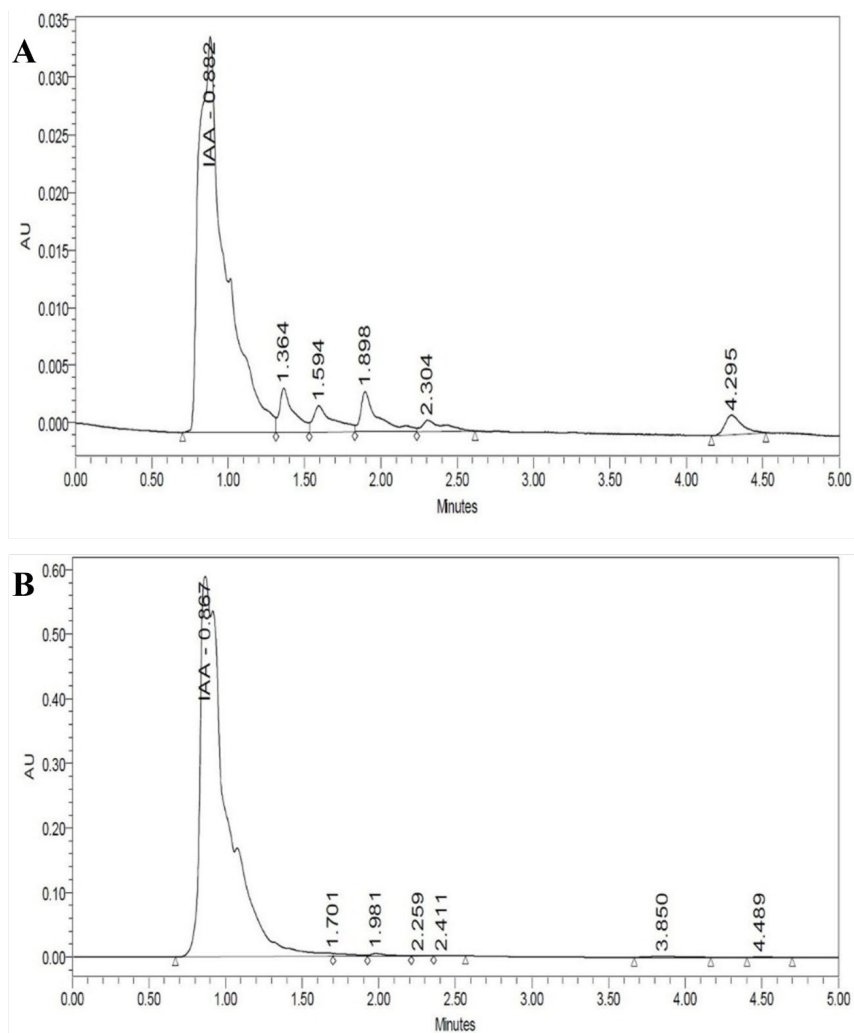


Figure 6. UPLC chromatogram of extracellular (A) and intracellular (B) IAA production in minimal media

4. Conclusions

Our study focused on isolating and characterizing endophytic bacterium from *Humulus lupulus*. Further study focused on LC-MS analysis of the dried extract of isolated bacterium, which revealed the presence of multiple bioactive compounds. Further analyses demonstrated that IAA was produced both intracellularly and extracellularly, in both complex and defined media, underscoring the metabolic versatility of the endophytic isolate. This indicates that both intracellular and extracellular IAA production contributed cumulatively to the overall yield. These findings suggest that endophytes from the medicinal plant *Humulus lupulus* can serve as eco-friendly sources of bioactive metabolites that possess agricultural and pharmaceutical relevance. Furthermore, in the future, whole-genome sequencing should be performed for the detection of the IAA production pathway

of the isolated strain. The study further paved the way for optimizing abiotic factors to increase the yield of IAA.

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6. Authors' Contributions

S.K: Writing – original draft, Experiment Validation, Software, Methodology, Investigation, Formal analysis, Data curation. A.M: Conceptualization and Supervision.

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7. Conflicts of Interest

The authors assert that there are no conflicts of interest among them

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