



Co-production of biosurfactant and indigo using wild-type *Acinetobacter* sp. isolated from soil

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Abstract

In this research, we investigated a naturally occurring, non-genetically modified strain of *Acinetobacter* sp., isolated from soil, which demonstrated the capability to produce both indigo and biosurfactant. During the screening, indole was used as the sole carbon source in M9 minimal medium. The strain exhibiting the most intense blue coloration was isolated and further analyzed. The blue dye extracted from the cell culture was confirmed as indigo through LC/MS analysis, showing an m/z value of 263.5, and H-NMR analysis. In LB medium, the wild-type *Acinetobacter* sp. strain produced approximately 6.8 mg/L of indigo from 1 mM indole. However, in M9 minimal medium, the production yield significantly increased to 45.5 mg/L. Notably, the isolated strain showed vigorous bubbling during growth, which could facilitate the transport of indole and indigo dye, both of which have low solubility, across cell membranes. Additionally, this strain was capable of degrading medium-chain C12 alkane efficiently. The whole genome was fully sequenced and analyzed for genes concerning biosurfactant and alkane metabolisms. In conclusion, utilizing a wild-type strain for indigo production offers a promising alternative to traditional chemical processes, addressing concerns related to genetically modified organisms in future applications.

Keywords *Acinetobacter* sp. · Indigo dye · Indole · Biosurfactant

1 Introduction

Indigo, traditionally extracted from *Indigofera* plants, is mainly used as a dye for denim fabric [1]. The growing demand for jeans has significantly increased the need for indigo dye, resulting in the widespread use of chemically synthesized indigo [2]. However, the production of synthetic indigo depends on petrochemicals and involves toxic reactants and catalysts, leading to considerable environmental pollution [3, 4]. Therefore, there is an increasing demand

for carbon-neutral and environmentally friendly processes. Over time, several eco-friendly and bioprocess methods have been developed to replace synthetic indigo, with enzyme-mediated synthesis from biomass and bioresources being particularly notable [4–7].

The enzymatic production of indigo utilizes indole or tryptophan as substrates (Fig. 1A). Indole is converted to 3-hydroxyindole (indoxyl) through monooxygenase-mediated C-3 hydroxylation, a process facilitated by tryptophanase when tryptophan is used [7]. This indoxyl is then oxidized and dimerized to form indigo. Several enzymes, such as flavin monooxygenase, toluene monooxygenase, naphthalene dioxygenase, and cytochrome P450 monooxygenase, can catalyze the indole-3-oxidation process [8–14]. Additionally, indole-3-acetate monooxygenase has also been identified for this reaction [15]. However, scaling up the production of high-concentration indigo through enzymatic conversion remains challenging, and the use of recombinant strains falls under the regulatory policies for genetically modified organisms (GMOs), complicating commercialization efforts.

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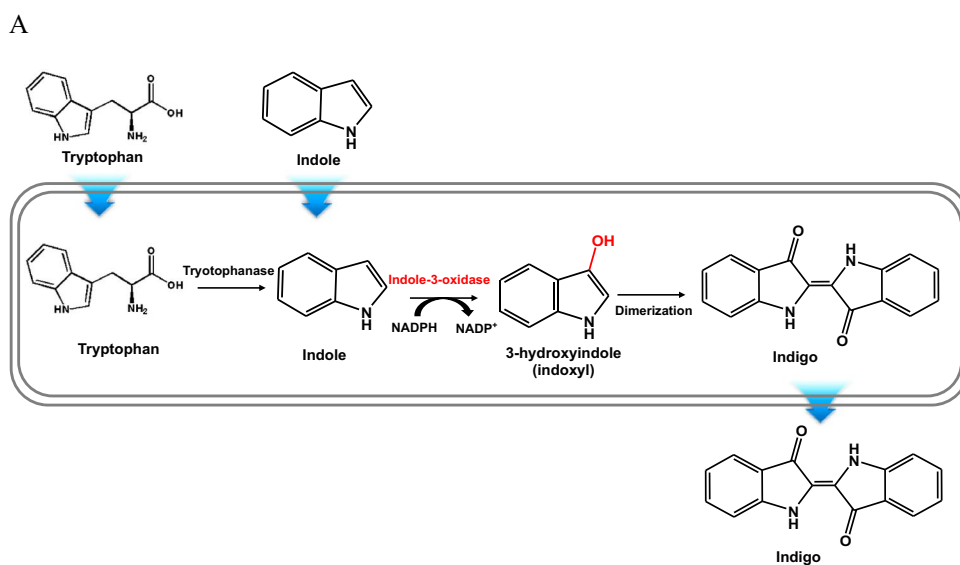
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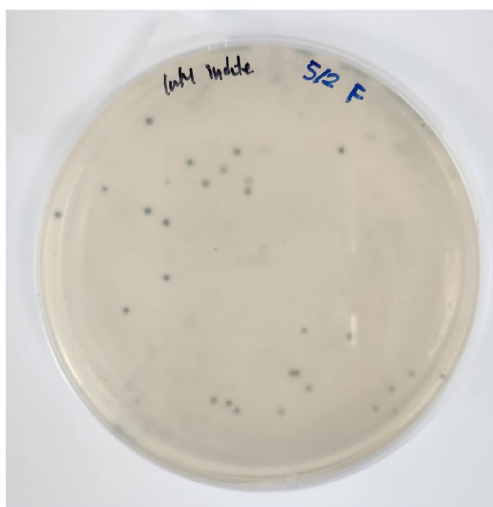
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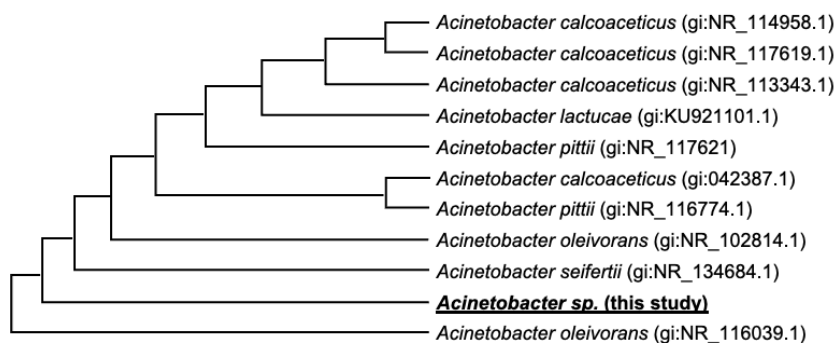
Fig. 1 **A** Synthetic pathway of indigo from indole. **B** Isolation of *Acinetobacter* species from soil source on indole agar plates, demonstrating distinct blue coloration. **C** 16S rRNA sequence analysis for strain identification revealing the isolated strain as *Acinetobacter* sp., closely related to *Acinetobacter oleivorans*



B



C



Another challenge in biological indigo synthesis is the natural production capabilities of wild-type strains. Strains such as *Pseudomonas*, *Streptomyces*, and *Acinetobacter* species possess monooxygenase enzymes in their

genomes that can oxidize indole [15–17]. This capability is easily detected through the formation of blue-colored colonies when indole is used as a substrate [8, 9]. Our research group previously isolated an indole-converting

microorganism from soil, identified as an *Acinetobacter* species, which produced up to 1.0 mg/L of indigo from 1 mM indole [15]. Sequence analysis revealed that the key enzyme involved in indole 3-oxidation was indole-3-acetate monooxygenase, encoded by the *icaA* gene. Heterologous expression of *icaA* resulted in a more than 20-fold increase in indigo production compared to the wild-type strain [15].

Despite these advancements, indigo production by wild-type strains has not exceeded 10 mg/L [15]. This low yield may be due to the intrinsic insolubility and toxic effects of indigo at high concentrations. Indigo tends to be highly insoluble, making it difficult to pass through the host membrane, often leading to truncation or aggregation between membranes [13]. Various strategies, including solubilization with detergents and reducing chemicals, have been employed to address this issue, achieving some success in increasing production yields [4]. However, there is still significant potential for improvement, particularly through host engineering strategies.

In this study, we isolated a new strain capable of converting indole into indigo from the soil and investigated its indigo production capabilities. The production varied with the nutrients present in the liquid culture. During incubation, the liquid culture exhibited abundant bubbling, identified as biosurfactant produced by *Acinetobacter*. We characterized the biosurfactant by measuring surface tension reduction and surfactant index. The study highlighted the relatively higher indigo production levels compared to other wild-type strains, considering the co-production of indigo and surfactant [18]. The strain's full genome was sequenced, and candidate genes responsible for indigo and biosurfactant production were identified.

Despite these promising results, several challenges remain for the industrialization of bio-based chemical production. Continuous research efforts are needed to transition from petroleum-based to bio-based eco-friendly methods. Specifically, the production of indigo and biosurfactants by microorganisms requires further advancements in host and process engineering [3, 4]. This study provides valuable insights into the co-production of biosurfactants with other valuable biochemicals, contributing to future advancements in this field.

2 Materials and methods

2.1 Bacterial strains and chemicals

Acinetobacter strains were sourced from soil samples collected from a farmland in Hamyang, Korea, following a fire incident. Indole, indigo, indirubin, ethyl acetate (EA),

hexadecane, and dodecane were procured from Sigma-Aldrich Korea. M9 minimal media, Luria–Bertani (LB), and Terrific Broth were obtained from Difco™ (Difco Korea).

2.2 Strain isolation and gene annotation

The soil samples were diluted with autoclaved distilled water and allowed to settle for a day. Concurrently, M9 minimal medium agar plates supplemented with 1 mM indole were prepared. 0.1 mL of the diluted soil samples were spread on these agar plates and incubated at 30 °C for one day [15]. Blue colonies were visually identified, isolated, and streaked onto new agar plates to obtain individual colonies.

2.3 Production of indigo and biosurfactant

The initial inoculation was done in LB broth taking single colony from LB agar plate, followed by shaking at 200 rpm and 30 °C overnight. Subsequently, 1% (v/v) of the overnight culture was transferred into 25 mL portions of LB broth, M9 minimal media with 1% glucose, and M9 minimal media, each supplemented with 1 mM indole [4, 15]. These cultures were incubated for 24 h at 200 rpm and 30 °C. For biosurfactant analysis, the strain was incubated in LB medium for 24 h under the same conditions.

2.4 Extraction of indigo and biosurfactant

Samples were centrifuged at 8,500 rpm for 15 min at 4 °C. Indigo was extracted from the cells by vortexing with an equal volume of EA to the culture medium, followed by centrifugation at 8,500 rpm for 15 min at 25 °C [4, 15]. The blue supernatant was then dried in a fume hood. For the culture medium supernatant, the pH was adjusted to 2 using 6 M HCl and 5N NaOH, and incubated overnight at 4 °C. An equal volume of EA was added for extraction, followed by centrifugation at 8,500 rpm for 15 min at 25 °C. The supernatant was subsequently dried in a fume hood.

2.5 Quantitative analysis of the synthesized indigo dye

Indigo quantification was performed using high-performance liquid chromatography (HPLC) [4, 15]. The HPLC system, equipped with a UV detector and a reverse-phase C18 column (Zorbax Extend-C18; 250 mm × 4.6 mm, 3.5 μm, Agilent Technologies), utilized a gradient solvent system composed of water (solvent A), methanol (solvent B), and acetonitrile (solvent C) in a ratio of 19:80:1.

2.6 1H NMR, liquid chromatography electrospray ionization mass spectrometry (LC–ESI–MS/MS) analysis for structural identification of indigo

For qualitative analysis of indigo, both LC–ESI–MS/MS and a 600 MHz high-resolution NMR Spectrometer were used. LC–ESI–MS/MS analysis employed an Ultimate 3000 RS system (Thermo Fisher Scientific Inc.) with a binary gradient system. Chromatographic separation of the dye was carried out on a C18 column (Waters Cortecs C18; 2.1 mm × 150 mm, 1.6 μm) with a 2 μL injection volume. The solvents used were water with 0.1% formic acid and methanol with 0.1% formic acid. *m/z* values were detected using an LTQ mass spectrometer (Thermo Fisher Scientific Inc.). For 1H NMR spectra, an AVANCE 600 instrument was utilized.

2.7 Physical properties of biosurfactant by emulsifying activity and surface tension

The emulsifying activity of the biosurfactant was determined by calculating the emulsion index (E_{24}) [18]. The cell-free supernatant was mixed with hexadecane in equal volumes and vortexed for 1 min. After 24 h at room temperature, the emulsification index (E_{24}) was calculated using Eq. (1), dividing the height of the emulsion layer by the total height of the liquid column. Measurements were performed in triplicate, and results were expressed as mean ± standard deviation.

$$\text{Emulsification index } E_{24} = \frac{\text{Height of the emulsion layer}}{\text{Total height of the liquid column}} \times 100\% \quad (1)$$

Surface tension of the supernatant was measured using a KSV Model Sigma 702 instrument (Biolin Scientific).

2.8 Dodecane biodegradability

For inoculum preparation, the strain was initially inoculated in LB broth and shaken at 200 rpm and 30 °C overnight. 1% (v/v) of the overnight culture was transferred to 100 mL of TB medium with 1 mM indole and incubated for 48 h at 200 rpm and 30 °C. After centrifugation (8500 rpm, 4 °C, 15 min), the cells were washed twice with 0.1 M potassium phosphate buffer (pH 7.0). Cell density was adjusted to OD 20, and cells were resuspended in the same buffer containing 1% (v/v) dodecane and 1 mM indole, then incubated at 30 °C and 200 rpm. Samples were collected at 0 and 24 h, vortexed with an equal volume of EA, and centrifuged. The organic solvent supernatant was analyzed using a 6500 GC gas chromatography system (Younglin, Anyang, South Korea), with

separation on an Agilent J&W GC column (CP-Sil 8 CB amine, 30 m, 0.25 mm i.d., 0.25 μm film thickness). The column temperature was initially set at 130 °C, raised to 230 °C at 20 °C/min, and held for 3 min. Nitrogen served as the carrier gas at 2 mL/min flow, with a 10:1 split ratio. Injection and detection temperatures were set at 330 °C and 300 °C, respectively.

3 Results and discussion

3.1 Screening and isolation of indigo and biosurfactant co-producing bacteria

Microorganisms were screened based on colony color, specifically targeting those the colonies that exhibited a blue hue. The strains displaying blue hue coloration were isolated and re-cultivated on indole-containing plates to confirm their capability to convert indole (Fig. 1B). The isolated strain exhibited a pronounced dark blue color, and a single colony was selected for further examination. 16S rRNA sequencing revealed that this strain belongs to the *Acinetobacter* genus, closely related to *Acinetobacter oleivorans* (Fig. 1C).

3.2 Structural analysis of isolated blue coloration dye

The structure of the blue dye was identified using LC–ESI–MS/MS and 1H NMR analyses. LC–ESI–MS/MS results showed 5 to 6 major metabolites in the full scan chromatogram. A peak with a retention time of 30.2 min corresponded to a mass value of 263.5 *m/z*, matching the molecular weight of indigo (Fig. 2A). Comparative analysis with chemically synthesized indigo showed a mass value of 263.4 *m/z* and fragment ion patterns at 239.7 and 217.6 *m/z* (Fig. 2B), confirming that the indole metabolite produced by the isolated *Acinetobacter* sp. was indigo dye.

1H NMR analysis further confirmed the indigo structure (Fig. 2C). Peaks at 2.50 ppm and 2.52 ppm were associated with dimethyl sulfoxide (DMSO) solvent, while peaks in the ranges of 6–7 ppm and 10 ppm indicated resonances for the N–H in indole and a 4-spin AA'BB' spectrum of indigo, respectively. The proton of the N–H in indigo appeared as a single peak at 10.5 ppm. Peaks at 7.51 ppm corresponded to protons H4 and H4' in the indole structure, and peaks at 7.28 ppm were attributed to protons H7 and H7', both doublets. Peaks at 7.47 and 7.02 ppm corresponded to protons H5, H5' and H6, H6', respectively, both triplets. These 1H NMR results were consistent with previously obtained data, confirming the blue dye as indigo synthesized by the wild-type *Acinetobacter* sp.

Fig. 2 **A** Separation of indigo in LC–ESI–MS/MS analysis. The blue dye was observed at a retention time of 30.2 and showed an exact match with the standard indigo chemical. **B** Mass analysis of the blue dye showed a distinct 263.5 m/z value which corresponds well with that of the indigo standard chemical. **C** H-NMR analysis of the produced indigo. LC–ESI–MS/MS: liquid chromatography electrospray ionization mass spectrometry

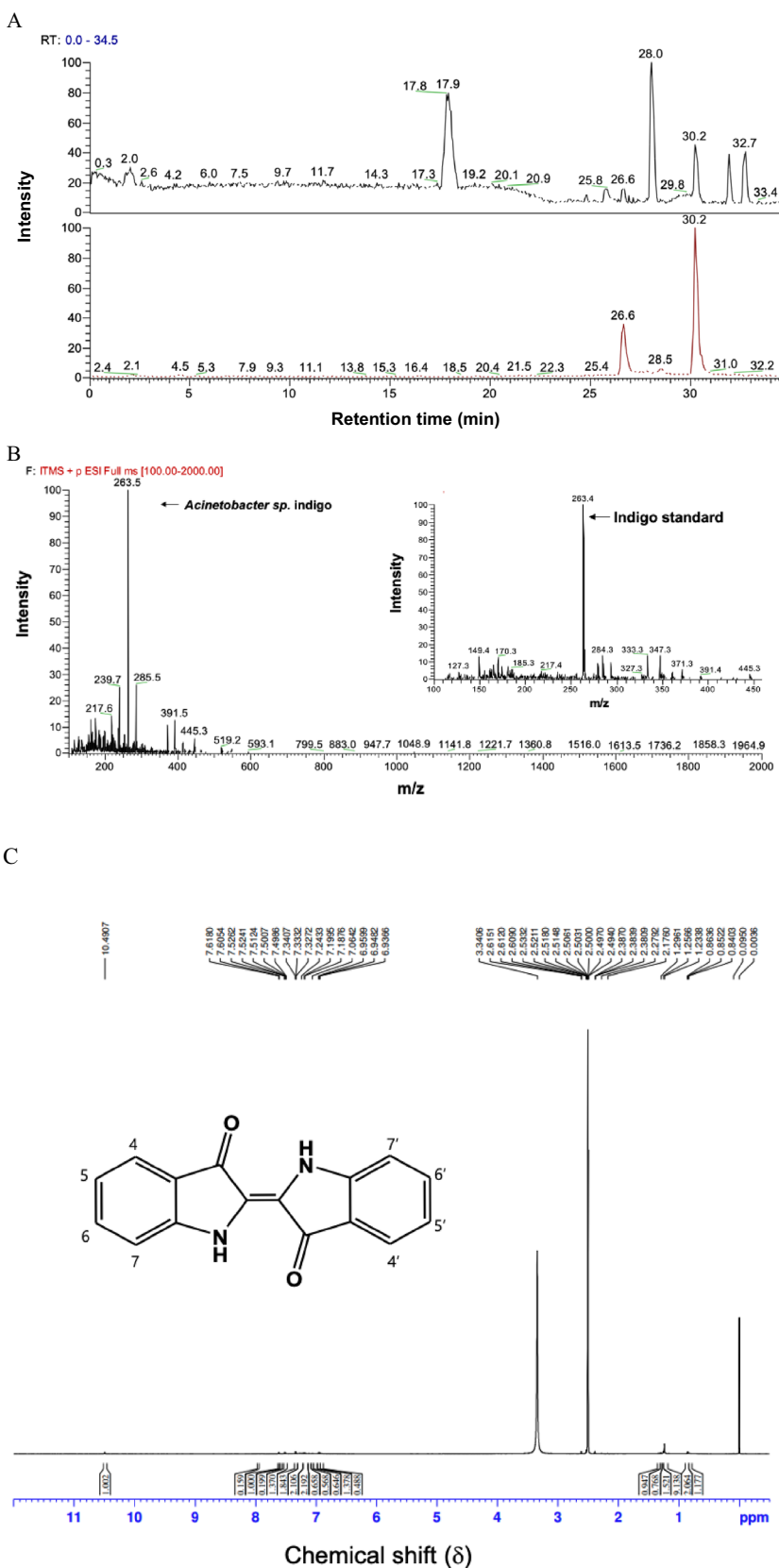
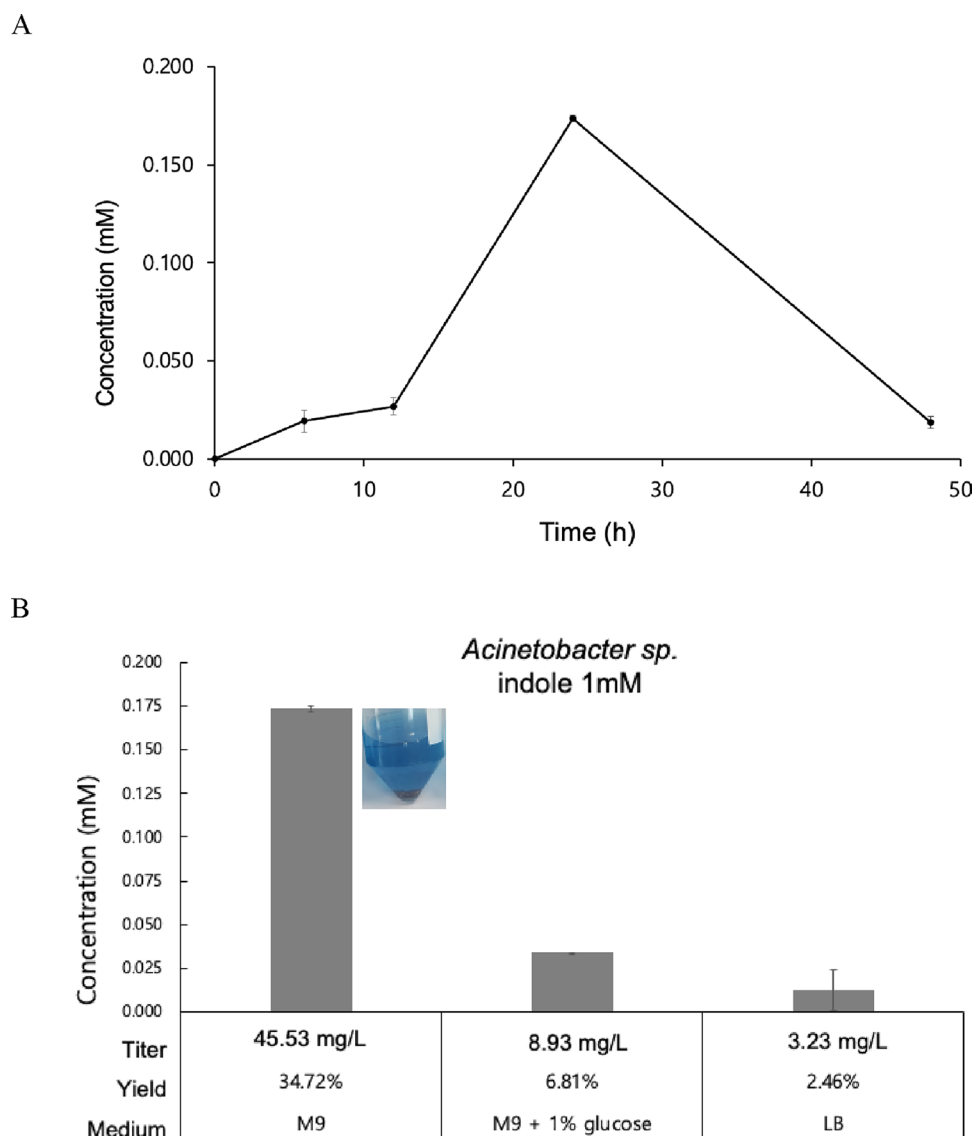


Fig. 3 **A** Time-dependent bio-indigo production profile by wild-type *Acinetobacter* strain. 1.0 mM indole was used for substrate. **B** Production of indigo using *Acinetobacter* strain from the biotransformation of 1.0 mM indole in M9 and LB medium

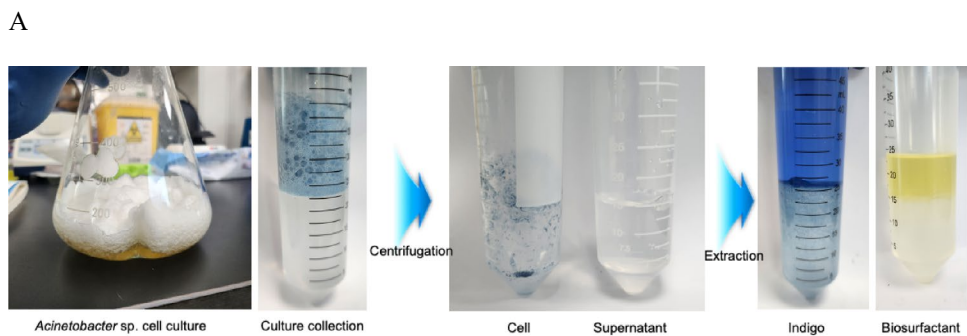


3.3 Production of indigo dye in M9 minimal and LB complex medium

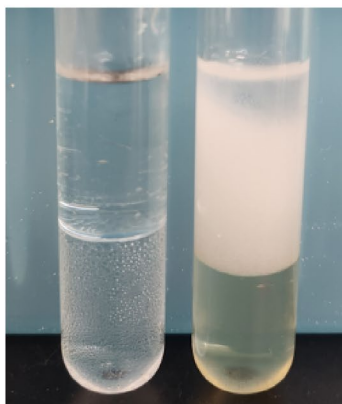
The indigo production by wild-type *Acinetobacter* sp. was evaluated in indole-supplemented M9 minimal medium with and without 1.0% glucose, as well as in LB complex medium. The highest indigo production titer was observed at 24 h and gradually decreased thereafter (Fig. 3A). In LB medium, indigo production did not exceed 3.5 mg/L, with a yield of around 2.5%, whereas in M9 medium without glucose, the highest indigo production of 45.5 mg/L was achieved

(Fig. 3B). The addition of glucose reduced indigo production by 8.9 mg/L, potentially due to lower indole uptake or repression of indole-3-oxidase expression despite the stable supply of NADPH from glucose metabolism. The blue coloration of the culture supernatant after centrifugation is shown in Fig. 4A (insert). Additionally, the cell pellet also exhibited blue coloration, suggesting partial secretion and intracellular accumulation of indigo.

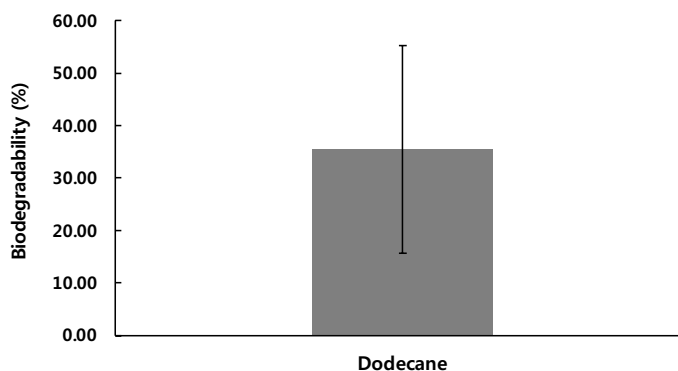
Fig. 4 **A** Culture broth of *Acinetobacter* sp. and bubbles observed during incubation. The pellet collected by centrifugation and supernatant after separation showed distinct indigo coloration. The biosurfactant was isolated from LB broth culture. **B** Measurement of emulsifying index by the generated *Acinetobacter* surfactant. **C** Dodecane degradation by isolated *Acinetobacter* strain



B



C

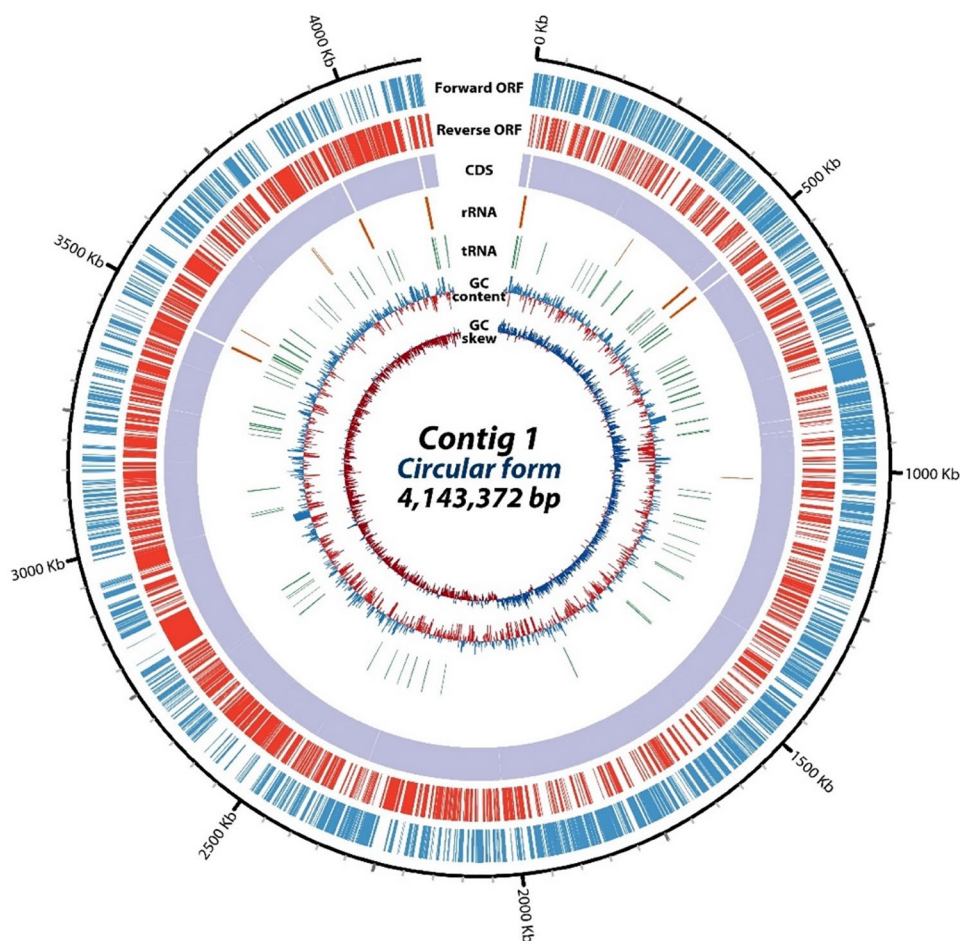


3.4 Co-production of biosurfactant by isolated *Acinetobacter* sp. and biosurfactant characterization

In addition to indigo production, abundant foam formation in the culture broth suggested biosurfactant production (Fig. 4B). Previous studies have reported biosurfactant production by *Acinetobacter* strains isolated from petroleum-contaminated soils with production around 0.52 g/L

[18, 19]. These biosurfactants can decrease surface tension, potentially affecting the production of hydrophobic metabolites by altering cell membrane permeability. The isolated *Acinetobacter* sp. reduced surface tension from 72.7 mN/m to 56.4 mN/m \pm 5.3, and exhibited an emulsification index of 52.2 \pm 7.5% in hexadecane (Fig. 4B). This strain exhibited higher indigo production compared to other wild-type strains, possibly due to simultaneous biosurfactant production, which could solubilize indole

Fig. 5 Full genome sequencing was completed and genes were annotated completely



and indigo through micelle formation, facilitating indole uptake and indigo release via permeabilized membranes.

3.5 Alkane biodegradability by *Acinetobacter* sp.

The alkane biodegradability of the strain was investigated, given that biosurfactant-producing strains often degrade crude oil. *A. oleivorans* DR1, a closely related species, is known for degrading crude oil and alkanes with carbon chain lengths from C₁₂ to C₃₀ [20]. The *Acinetobacter* sp. successfully degraded 1% dodecane with 35.5% efficiency

(Fig. 4C). During the whole-cell reaction, biosurfactants generated during growth were almost completely utilized, suggesting that the biosurfactant enhances alkane solubility, improving uptake and degradation by the strain.

3.6 Whole genome sequencing of isolated *Acinetobacter* sp. and candidate genes involved in indigo and surfactant synthesis

The whole genome of the strain was sequenced, identifying key genes potentially involved in surfactant production and

Table 1 Genes involved in the bio-surfactant production in *Acinetobacter* strains

Type	Gene	Description	Length (bp)
Aminolipids	<i>proA</i>	Gamma-glutamyl phosphate reductase	1,365
	<i>gdhA_1</i>	Glu/Leu/Phe/Val dehydrogenase	1,275
	<i>gdhA_2</i>	NADP-specific glutamate dehydrogenase	1,404
Glycolipids	<i>otsA</i>	Alpha, alpha-trehalose-phosphate synthase (UDP-forming)	1,434
	<i>otsB</i>	MULTISPECIES: trehalose-phosphatase	852
	<i>pckG</i>	MULTISPECIES: phosphoenolpyruvate carboxykinase (GTP)	1,833
	<i>ppc</i>	MULTISPECIES: phosphoenolpyruvate carboxylase	2,685
	<i>ppsA_1</i>	Phosphoenolpyruvate synthase	2,379

Table 2 Genes involved in the alkane metabolism of *Acinetobacter*

Gene	Description	Length (bp)
<i>adh1</i>	Iron-containing alcohol dehydrogenase	1,158
<i>adhA</i>	NAD(P)-dependent alcohol dehydrogenase	1,053
<i>ahr</i>	NAD(P)-dependent alcohol dehydrogenase	1,029
<i>adhB</i>	Iron-containing alcohol dehydrogenase	1,173
<i>alkB_1</i>	Alkane 1-monooxygenase	1,224
<i>alkB_2</i>	Alkane 1-monooxygenase	1,194
<i>dhaT</i>	Iron-containing alcohol dehydrogenase	1,185
<i>frmA</i>	S-(hydroxymethyl)glutathione dehydrogenase/class III alcohol dehydrogenase	1,110
<i>ssuD_1</i>	LLM class flavin-dependent oxidoreductase	1,197
<i>ssuD_2</i>	LLM class flavin-dependent oxidoreductase	1,134
<i>ssuD_3</i>	LLM class flavin-dependent oxidoreductase	1,089
<i>ssuD_5</i>	FMNH ₂ -dependent alkanesulfonate monooxygenase	1,176

alkane biodegradation (Fig. 5). Genes related to aminolipids (phosphate reductase, glutamate dehydrogenases) and glycolipids (trehalose phosphate synthase, phosphoenolpyruvate synthetase) were predicted (Table 1). *Acinetobacter* species typically produce rhamnolipids [21, 22]. According to a previous report, the biosurfactant produced by indigenous *Acinetobacter junii* was identified as a rhamnolipid type with C₂₆H₄₈O₉, C₂₈H₅₂O₉, and C₃₂H₅₈O₁₃ as the dominant components [21].

Genes encoding alcohol dehydrogenases, alkane-1-monooxygenases, flavin-dependent oxidoreductases, and alkanesulfonate monooxygenases, potentially responsible for alkane degradation, were also predicted (Table 2). Enzymes such as indole-3-acetate monooxygenase and alkane 1-monooxygenase are being investigated through heterologous expression in *Escherichia coli*.

4 Conclusion

The screening of *Acinetobacter* sp. capable of producing blue dye was performed using indole agar plates, identifying the strain's dual ability to produce indigo and biosurfactant. The wild-type *Acinetobacter* sp. strain produced approximately 6.8 mg/L of indigo from 1 mM indole in LB media, while in M9 minimal media, the yield significantly increased to 45.5 mg/L. A notable observation was the vigorous bubbling exhibited by the strain during growth, potentially facilitating the transport of indole and indigo dye, both of which have low solubility, across cell membranes. Additionally, the strain demonstrated the capability to degrade C12 medium chain alkane.

In conclusion, the utilization of a wild-type strain for indigo production and biosurfactant supply during biotransformation offers a promising platform for alternative chemical processes, addressing concerns related to GMOs in future applications. This finding holds significant potential for the large-scale production of indigo and biosurfactant, paving the way for commercialization in various biochemical production applications. [23].

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Statements Neither ethical approval nor informed consent was required for this study.

References

- Dutta S, Roychoudhary S, Sarangi BK (2017). 3 Biotech. <https://doi.org/10.1007/s13205-017-0923-2>
- Kaplan G, Seferoğlu Z (2023) The synthetic approaches for preparation of indigo and applications in denim industry. *Curr Org Synth* 20:361–364. <https://doi.org/10.2174/1570179419666220830091956>
- Yi C, Tan X, Bie B et al (2020) Practical and environment-friendly indirect electrochemical reduction of indigo and dyeing. *Sci Rep* 10:4927. <https://doi.org/10.1038/s41598-020-61795-5>
- Yuk Y, Jang JH, Park SA et al (2023) Production of bio-indigo dye by surmounting its physical and chemical insoluble nature. *Dyes Pigments* 218:111466. <https://doi.org/10.1016/j.dyepig.2023.111466>
- Choi KY (2021) Discoloration of indigo dyes by eco-friendly biocatalysts. *Dyes Pigments* 184:108749. <https://doi.org/10.1016/j.dyepig.2020.108749>

6. Lee J, Kim J, Song JE et al (2021) Production of Tyrian purple indigoid dye from tryptophan in *Escherichia coli*. *Nat Chem Biol* 17:104–112. <https://doi.org/10.1038/s41589-020-00684-4>
7. Namgung S, Park HA, Kim J et al (2019) Ecofriendly one-pot biosynthesis of indigo derivative dyes using CYP102G4 and PrnA halogenase. *Dyes Pigments* 162:80–88. <https://doi.org/10.1016/j.dyepig.2018.10.009>
8. Fabara AN, Fraaije MW (2020) An overview of microbial indigo-forming enzymes. *Appl Microbiol Biotechnol* 104:925–933. <https://doi.org/10.1007/s00253-019-10292-5>
9. Kim HJ, Jang S, Kim J et al (2017) Biosynthesis of indigo in *Escherichia coli* expressing self-sufficient CYP102A from *Streptomyces cattleya*. *Dyes Pigments* 140:29–35. <https://doi.org/10.1016/j.dyepig.2017.01.029>
10. Yin S, Li Y, Hou J (2024) Expression of the two-component regulator StyS/StyR enhanced transcription of the styrene monooxygenase gene styAB and indigo biosynthesis in *Escherichia coli*. *Enzyme Microb Technol* 174:110381. <https://doi.org/10.1016/j.enzmictec.2023.110381>
11. Ma L, Sun T, Liu Y et al (2023) Enzymatic synthesis of indigo derivatives by tuning P450 BM3 peroxygenases. *Synth Syst Biotechnol* 8:452–461. <https://doi.org/10.1016/j.synbio.2023.06.006>
12. Kim HJ, Ham S, Shin N et al (2024) Tryptophan-based hyperproduction of bioindigo by combinatorial overexpression of two different tryptophan transporters. *J Microbiol Biotechnol* 34:969–977. <https://doi.org/10.4014/jmb.2308.08039>
13. Han GH, Gim GH, Kim W et al (2012) Enhanced indirubin production in recombinant *Escherichia coli* harboring a flavin-containing monooxygenase gene by cysteine supplementation. *J Biotechnol* 164:179–187. <https://doi.org/10.1016/j.jbiotec.2012.08.015>
14. Choi HS, Kim JK, Cho EH et al (2003) A novel flavin-containing monooxygenase from *Methylophaga* sp strain SK1 and its indigo synthesis in *Escherichia coli*. *Biochem Biophys Res Commun* 306:930–936. [https://doi.org/10.1016/s0006-291x\(03\)01087-8](https://doi.org/10.1016/s0006-291x(03)01087-8)
15. Ahn S, Park S, Kumar P et al (2023) Bio-indigo production using wild-type *Acinetobacter* sp. and indole-3-acetate monooxygenase (*iacA*) expressed in *Escherichia coli*. *Biotechnol Bioprocess Eng* 28:281–288. <https://doi.org/10.1007/s12257-022-0163-0>
16. Mercadal JP, Isaac P, Siñeriz F et al (2010) Indigo production by *Pseudomonas* sp. J26, a marine naphthalene-degrading strain. *J Basic Microbiol* 50:290–293. <https://doi.org/10.1002/jobm.200900276>
17. Kim J, Lee PG, Jung EO et al (2018) In vitro characterization of CYP102G4 from *Streptomyces cattleya*: a self-sufficient P450 naturally producing indigo. *Biochim Biophys Acta Proteins Proteom* 1866:60–67. <https://doi.org/10.1016/j.bbapap.2017.08.002>
18. Chen J, Huang PT, Zhang KY et al (2012) Isolation of biosurfactant producers, optimization and properties of biosurfactant produced by *Acinetobacter* sp. from petroleum-contaminated soil. *J Appl Microbiol* 112:660–671. <https://doi.org/10.1111/j.1365-2672.2012.05242.x>
19. Bao M, Pi Y, Wang L et al (2014) Lipopeptide biosurfactant production bacteria *Acinetobacter* sp. D3–2 and its biodegradation of crude oil. *Environ Sci Process Impacts* 16:897–903. <https://doi.org/10.1039/c3em00600j>
20. Park C, Shin B, Jung J et al (2017) Metabolic and stress responses of *Acinetobacter oleivorans* DR1 during long-chain alkane degradation. *Microb Biotechnol* 10:1809–1823. <https://doi.org/10.1111/1751-7915.12852>
21. Dong H, Xia W, Dong H et al (2016) Rhamnolipids produced by indigenous *Acinetobacter junii* from petroleum reservoir and its potential in enhanced oil recovery. *Front Microbiol* 7:1710. <https://doi.org/10.3389/fmicb.2016.01710>
22. Zhu P, Zhang S, Kumar R et al (2022) Rhamnolipids from non-pathogenic *Acinetobacter calcoaceticus*: bioreactor-scale production, characterization and wound healing potency. *N Biotechnol* 67:23–31. <https://doi.org/10.1016/j.nbt.2021.12.001>
23. Kadam V, Dhanorkar M, Patil S et al (2024) Advances in the co-production of biosurfactant and other biomolecules: statistical approaches for process optimization. *J Appl Microbiol* 135:lxae025. <https://doi.org/10.1093/jambio/lxae025>

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