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# Elimination of ineffective inorganic salt component in medium for indole-3-acetic acid synthesis by *Serratia plymuthica* UBCF_13 and its effect on the growth of chili seedlings

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**ABSTRACT** Indole-3-acetic acid (IAA) is an essential phytohormone that controls a variety of plant growth mechanisms. Bacteria can produce IAA to stimulate plant growth, with its production influenced by the culture conditions. *Serratia plymuthica* UBCF_13 is recognized as an IAA-producing bacterium, exhibiting maximum IAA production in a yeast medium comprising yeast extract, sucrose, $K_2HPO_4$, $MgSO_4$, NaCl, and $CaCO_3$. However, prior studies optimizing individual inorganic salt components indicated minimal impact on IAA synthesis within this medium. This study aimed to eliminate the unnecessary inorganic salt components and the medium was then applied to investigate the IAA biosynthesis pathway and the plant growth-promoting assay. The elimination assay consisted of yeast sucrose medium devoid of $K_2HPO_4$, $MgSO_4$, NaCl, or $CaCO_3$, and yeast sucrose medium containing only $MgSO_4$ and $CaCO_3$. Various indole compounds were then added to the revised medium composition to investigate the IAA biosynthesis pathway of UBCF_13 using high-performance liquid chromatography (HPLC). Furthermore, the effect of UBCF_13 culture supernatant, cultivated in the new medium, on chili plant growth was evaluated. The highest IAA production (138.8 µg/mL) was observed in the yeast sucrose with $CaCO_3$ and $MgSO_4$ (elimination of $K_2HPO_4$ and NaCl). The presence of indole-3-acetamide (IAM) compound from the medium extracts, supplemented with multiple indole compounds, revealed that UBCF_13 may use the IAM pathway. The application of UBCF_13 supernatant enhanced the shoot, root length, fresh weight, and germination time of chili seeds by 37.7%, 49.3%, 204.3%, and 38.6%, respectively. This study demonstrated that eliminating $K_2HPO_4$ and NaCl provided a new culture medium composition conducive to IAA production by UBCF_13. Moreover, the UBCF_13 extract has the potential to promote plant growth.

**KEYWORDS** Biofertilizer; IAA; Inorganic salt; Medium optimization; Plant growth promoting bacteria

## 1. Introduction

Indole-3-acetic acid (IAA) is a regulating growth that controls a variety of plant growth mechanisms. Biofertilizers can be added to maintain the supply of IAA in plants. Biofertilizer contains bacteria that can not only produce IAA to improve plant growth but also minimize the negative effect of chemical fertilizers (Emami et al. 2019; De Fretes et al. 2021; Daniel et al. 2022; Hossain et al. 2023). Studies regarding the effect of IAA-producing bacteria to promote plant growth have been widely reported (Suresh et al. 2019; Chopra et al. 2020; Kaur and Manhas 2022; Niu et al. 2022). For example, inoculation of endophytic bacteria with IAA-producing ability showed a positive response to the growth of sweet sorghum (De Fretes et al. 2021).

The quantity of IAA that bacteria synthesize is significantly influenced by the culture conditions; therefore, optimizing the components of the culture medium is essential to increasing bacterial IAA production (Bunsangiam et al. 2021; Baliyan et al. 2021). *Serratia plymuthica* UBCF_13 is a phylloplane isolate capable of producing IAA (Aisyah et al. 2019; Yusfi et al. 2021). The optimization of IAA production by UBCF_13 has been investigated based on culture time, medium type, nitrogen source, carbon source, and mineral composition. Maximum IAA production of UBCF_13 has been obtained in yeast sucrose medium with 300 µg/mL tryptophan and culture durations of 9 h and 21 h based on the colorimetric and high-performance liquid chromatography (HPLC) assays, respectively (Yusfi et al. 2021, 2022, 2023a). According to the optimization of in-

dividual inorganic salt components from a previous study, IAA production in a culture medium with the addition of $K_2HPO_4$ and NaCl addition was low, 1.4 and 0 µg/mL, respectively (Yusfi et al. 2023a). In term of low IAA concentration in the medium with $K_2HPO_4$ and NaCl highlights, investigation the effect of eliminating these minerals is required.

Culture medium optimization is a crucial step in ensuring greater production at a lower cost. A culture medium containing no mineral salts was found to have the best effect on the growth of *Pseudoxanthomonas indica* H32. (Morales-Borrell et al. 2020). The micronutrients in the culture medium have previously been depleted to assess the significance of certain micronutrients on *Microcystis aeruginosa* growth (Facey et al. 2022). Thus, the culture medium optimization process entails both adding and eliminating components. The removal of these unnecessary components may also result in the formation of a novel and most effective medium composition for IAA production.

In the present study, we eliminated each inorganic salt component from the yeast sucrose medium. Then several indole compounds could be supplemented to the culture medium to ensure the IAA synthesis pathway of UBCF_13. The application of indole compounds as precursors has been examined in several IAA-producing microorganisms to investigate the IAA biosynthesis pathway (Lin et al. 2018; Sun et al. 2018; Bunsangiam et al. 2019; Leontovyčová et al. 2020; Jahn et al. 2021). Additionally, the plant growth-promoting assay was also conducted by soaking the chili seeds with UBCF_13 culture and supernatant. In UBCF_13, however, neither the application of indole compounds as precursors nor the plant growth-promoting assay in yeast sucrose medium has previously been studied. Therefore, this study aimed to eliminate the ineffective components and then the medium was applied to investigate the IAA biosynthesis pathway and the plant growth-promoting assay.

## 2. Materials and Methods

### 2.1. Isolate cultivation

*Serratia plymuthica* strain UBCF_13 (Collection of Biotechnology Laboratory, Universitas Andalas) was grown for 24 h at 28 °C on Luria Bertani medium.

### 2.2. Inorganic salt component reduction

Table 1 shows the inorganic salt component reduction treatments used.

The experiment used a completely randomized design in triplicate (5 unit treatments with 3 replicates). All treatments were incubated in a shaking incubator at 160 rpm, 28 °C for 9 h and 21 h. The measurement of optical density and IAA concentration referred to (Yusfi et al. 2022) using a UV spectrophotometer at 600 and 530 nm, respectively.

**TABLE 1** Inorganic salt component reduction treatments.

| Treatment code | Description of composition |
|---|---|
| (-) $K_2HPO_4$ | 1 mg/mL yeast extract + 10 mg/mL sucrose + 1 mg/mL $CaCO_3$ + 0.1 mg/mL NaCl + 0.2 mg/mL $MgSO_4$ + 300 µg/mL tryptophan |
| (-) $MgSO_4$ | 1 mg/mL yeast extract + 10 mg/mL sucrose + 1 mg/mL $CaCO_3$ + 0.1 mg/mL NaCl + 0.5 mg/mL $K_2HPO_4$ + 300 µg/mL tryptophan |
| (-) NaCl | 1 mg/mL yeast extract + 10 mg/mL sucrose + 1 mg/mL $CaCO_3$ + 0.2 mg/mL $MgSO_4$ + 0.5 mg/mL $K_2HPO_4$ + 300 µg/mL tryptophan |
| (-) $CaCO_3$ | 1 mg/mL yeast extract + 10 mg/mL sucrose + 0.1 mg/mL NaCl + 0.2 mg/mL $MgSO_4$ + 0.5 mg/mL $K_2HPO_4$ + 300 µg/mL tryptophan |
| $MgSO_4$ and $CaCO_3$ | 1 mg/mL yeast extract + 10 mg/mL sucrose + 0.2 mg/ml $MgSO_4$ + 1 mg/mL $CaCO_3$ + 300 µg/mL tryptophan |

### 2.3. Application of several indole compounds as precursors

To verify the IAA biosynthesis pathway of UBCF_13, several indole compounds were used to replace tryptophan as the precursor in optimized medium culture. UBCF_13 was inoculated to yeast sucrose medium with $CaCO_3$, $MgSO_4$, and 300 µg/mL precursor. The precursors consist of tryptophan (TRP), indole-3-acetonitrile (IAN), indole-3-pyruvic acid (IPyA), tryptamine (TAM) and indole-3-acetamide (IAM). The cultures were incubated at 28 °C in a shaking incubator at 160 rpm for 21 h. The cultures were then used for HPLC analysis to study indole compounds' applications as precursors.

### 2.4. IAA crude extraction and HPLC analysis

The cultures were centrifuged at 10,000 rpm for 10 minutes at 4 °C. The supernatant (15 mL) was combined with 1N HCl (0.3 mL) to obtain a pH of 2.7. Afterward, the extraction was then performed twice using a double volume of ethyl acetate (99.5%) in a separator funnel. The ethyl acetate fraction was evaporated using a rotary evaporator (Heidolph, Germany), after which the extract was diluted in methanol (HPLC grade, 99.8%) and kept at –20 °C (Rupal K et al. 2020). The crude extract was evaluated by HPLC (Shimadzu 10A-Tvp HPLC system, Japan) using a Shim-pack MRC-ODS RP - 18 column (Shimadzu, Tokyo, Japan). The mobile phase was acetonitrile:water:acetic acid (40:60:0.01,v/v) at a flow rate of 1.0 mL $min^{-1}$. The run time was 30 minutes (Sun et al. 2018). The indole compounds were detected at 280 nm. Comparisons were then made with the retention time and standard curve of indole standards from Yusfi et al. (2022) to verify and quantify the indole compound.

### *2.5. Application of UBCF_13 extract to chili*

Previous studies have shown that the application of UBCF_13 culture had significant effects on the growth of various Solanaceae plants, particularly chili commodity (Aisyah et al. 2019). Therefore, in this study, we investigated the effect of IAA produced in the novel medium on chili germination. The UBCF_13 culture and supernatant were applied to chili seeds with different immersion durations. UBCF_13 was incubated in yeast sucrose medium with $CaCO_3$, $MgSO_4$, and 300 µg/mL tryptophan for 21 h. Chili seeds var. Lotanbar (Lotanbar, West Sumatra) were surface sterilized with different concentrations of sodium hypochlorite (NaOCl) (15%, 10%, and 5% (v/v)) for 10 min and before rinsing three times with sterilized distilled water. The sterilized seeds were then treated with sterilized distilled water (as a control), UBCF_13 culture (no dilution, 1:2 dilution, and 1:4 dilution) and UBCF_13 culture supernatant (no dilution, 1:2 dilution and 1:4 dilution) combined with different soaking times (30 min, 45 min, and 60 min). This experiment used a two-way factorial in a completely randomized design (7×3) in triplicate with 25 seeds per replication. The first factor was the seed soaking medium (control/water, culture and supernatant with dilution) and the second factor was soaking time. The IAA content on bacterial supernatant was also calculated using the colorimetry method according to Gordon and Weber (1951). The seeds were then placed in a jar (8 cm diameter and 12 cm height) containing water agar (10 g/L) at 28 °C. After 4 weeks, the fresh weight and germination time were recorded. The root and shoot lengths were also measured using the ImageJ program.

### *2.6. Statistical analysis*

The data were analyzed using analysis of variance (ANOVA). A one-way ANOVA was used to analyze the growth and IAA content of the inorganic salt component reduction study. A two-way ANOVA was used to analyze the data on the germination of chili seeds. Duncan's Multiple Range Test (DMRT) (significance = 0.05) was employed for mean separation using SPSS version 24.0 software.

## 3. Results and Discussion

### *3.1. Effect of reducing inorganic salt component on bacterial density and IAA production*

Based on Figure 1, the lowest optical density was observed in the medium with $MgSO_4$ reduction for both incubation times. In contrast, the maximum optical density (1.5) was obtained in a medium from which $CaCO_3$ had been eliminated.

The synthesis of IAA by UBCF_13 was unaffected by the removal of NaCl and $K_2HPO_4$ from the yeast sucrose medium. This result was supported by the development of a dark red color and the high IAA content obtained from this treatment (Figure 2). However, the removal of $MgSO_4$ from the yeast sucrose significantly reduced the IAA production of UBCF_13. This finding was confirmed by the formation of a pale pink color and the low IAA content at 9 h (6.9 µg/mL) (Figure 2a and 2c) and 21 h of incubation (62.9 µg/mL) (Figure 2b and 2d). The highest IAA production was 138.8 µg/mL which was found in yeast sucrose medium with $MgSO_4$ and $CaCO_3$ at 21 h of incubation (Figure 2).

### *3.2. Application of several indole compounds as precursor*

In the optimized medium, several indole compounds were utilized as precursors to determine the IAA biosynthetic pathway that was applied in UBCF_13. IAA was detected in the crude extract of all treatments using HPLC (Figure 3). TRP, IAM, and IAA were detected in the medium with the addition of TRP, IAM and TAM (Figure S1A - C). An IAM peak was also observed in the medium with IAN addition, although no TRP was detected in this medium (Figure S1D). TRP was observed in the medium with the addition of IPyA; however, no IAM peak was detected (Figure S1E). Based on Figure 3, the maximum TRP and IAA concentration was found in the medium with the addition of TRP at 175.8 µg/mL and 566.2 µg/mL, respectively. Meanwhile, in the medium with TRP addition, the highest IAA production was found in the medium with the addition of IAM. Here, the IAM concentration also increased significantly. IAM was additionally obtained in the medium with TAM and IAN at 28.5 µg/mL and 47.5 µg/mL, respectively. Interestingly, a similar IAA concentration was observed at about 21 µg/mL in the medium with IAN, TAM, and IPyA.

### *3.3. Application of UBCF_13 extract to chili*

The effect of UBCF_13 culture and supernatant on the germination of chili seeds was evaluated. Compared to the control seeds, seeds treated with the supernatant of UBCF_13 culture showed an increase in shoot and root length. However, seeds treated with UBCF_13 culture showed inhibited radicle and plumule development (Figure 4a). The root length, shoot length, and fresh weight of the seeds treated with the UBCF_13 culture supernatant showed significant increases of 49.3%, 37.7% and 204.3% respectively, compared to the control (Figure 4b(a-c)). The application of UBCF_13 culture supernatant also accelerated the seed germination time by 38.6% (Figure 4b(d)). In contrast, the application of UBCF_13 culture did not have a positive effect on the shoot length, root length, fresh weight, or germination time of the seeds. The root length and seed germination time were not influenced by supernatant dilution, however, the shoot length and fresh weight were significantly enhanced by the 1:2 and 1:4 dilution of supernatant (Figure 4b(b-c)). Overall, the soaking time of seeds on culture and supernatant of UBCF_13 did not significantly affect the growth of the seedlings, except for the shoot length, which was significantly increased by 30 mins of soaking time (Figure 4b(b)).

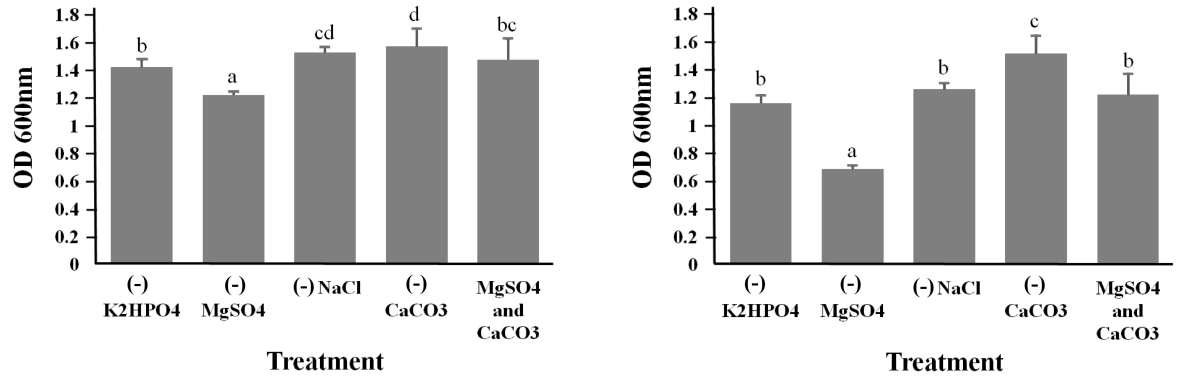



**FIGURE 1** Optical density of UBCF_13 based on inorganic salt component reduction at 9 h of incubation (a) and 21 h of incubation (b). Values were the means of three replicates ±SD. Lowercase letters represent significant differences among treatments by DMRT (alpha = 0.05%).

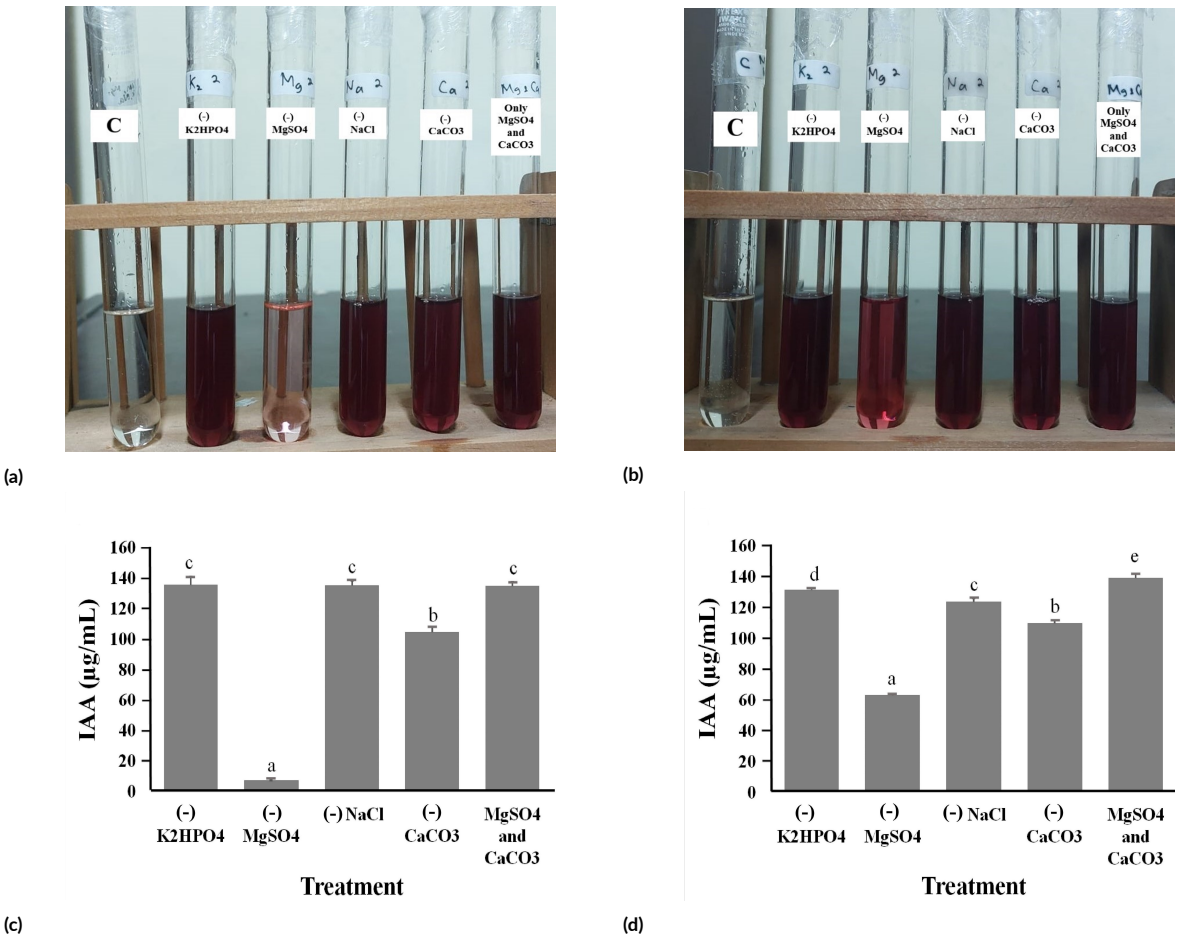



**FIGURE 2** Red color formation after salkowski reagent addition to the supernatant of samples with 9 h of culture time (a) and 21 h of culture time (b) (C=control). IAA production of UBCF_13 based on inorganic salt component reduction on 9 h of incubation (c and 21 h of incubation (d). Values were the means of three replicates ±SD. Lowercase letters represent significant differences among treatments by DMRT (alpha = 0.05%).

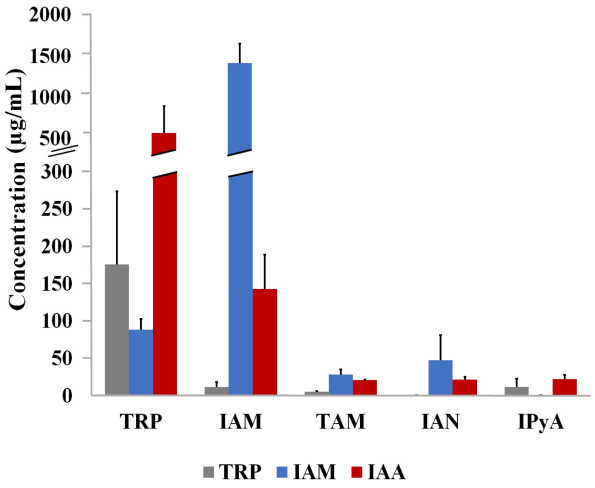



**FIGURE 3** The indole compound concentration detected from the crude extract of culture medium with the addition of different precursors (TRP, IAM, IAN, TAM, and IPyA). Values were the means of three replicates ±SD.

### *3.4. Discussion*

#### 3.4.1 Effect of reducing inorganic salt component on bacterial density and IAA production

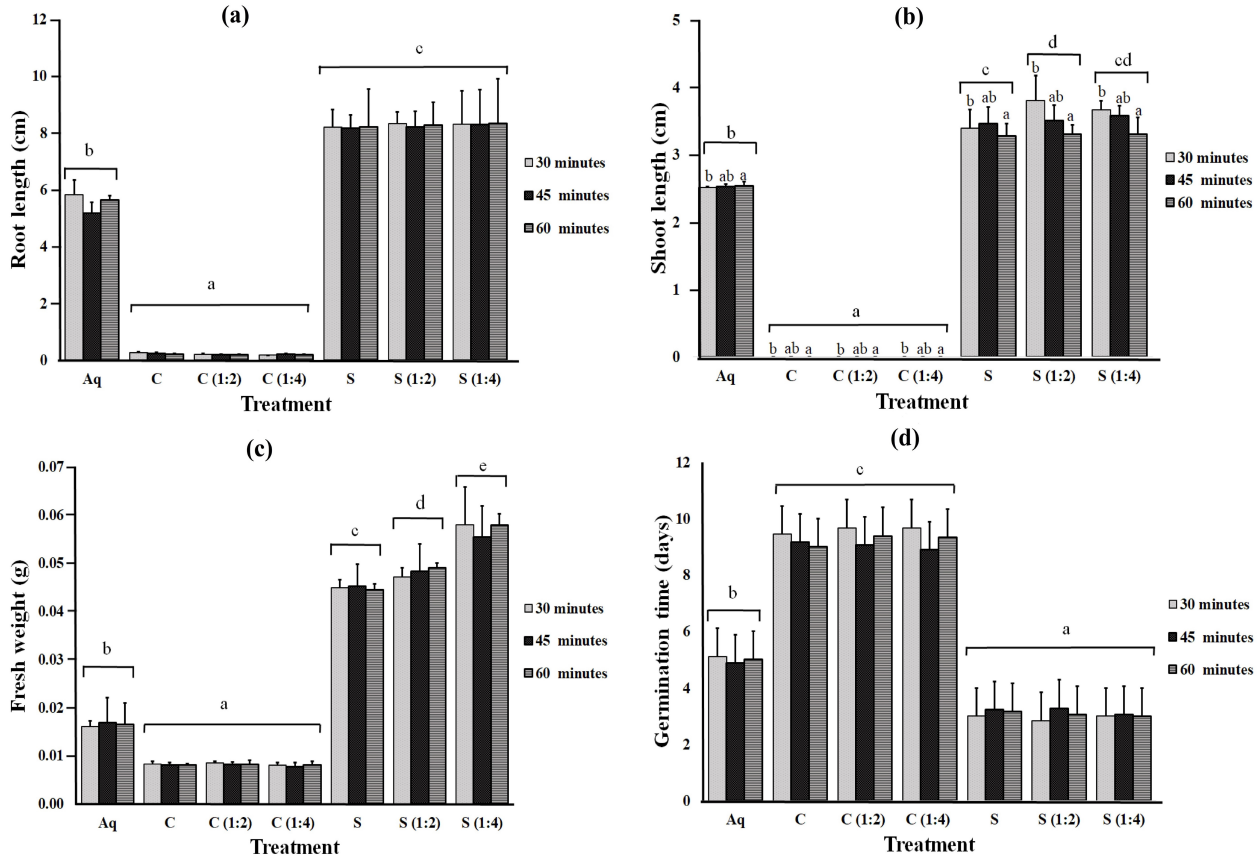
A previous study identified yeast sucrose with complete inorganic salt as the best media for IAA production by UBCF_13. Meanwhile, $MgSO_4$ and $CaCO_3$ were recorded as the most influential inorganic salts to produce IAA (Yusfi et al. 2023a). This study endeavored to minimize the yeast sucrose medium component by eliminating unnecessary inorganic salts. The effect of eliminating inorganic salt on bacterial density and IAA concentration was analyzed. Eliminating $MgSO_4$ reduced the bacterial density and IAA production in both 9 h and 21 h of incubation (Figures 1 and 2). This result aligns with the previously cited study, which found that UBCF_13 grown in medium with $MgSO_4$ as the sole inorganic salt had the highest IAA content and maximum bacterial density (Yusfi et al. 2023a,b). Every bacterium has a unique inorganic salt that can encourage the formation of IAA. Similar to UBCF_13, *Kosakonia pseudosacchari* TCPS-4 was found to demonstrate the highest IAA production in a culture medium with the addition of $MgSO_4$ (Chaudhary et al. 2021). Other strains, namely *Bifidobacterium adolescentis* Z25 and *Bacillus subtilis* MF447840.1, were also found to grow best in the presence of $MgSO_4$ (Sandhibigraha et al. 2020; Cui et al. 2021). Magnesium plays a vital role as a cofactor for nucleotide triphosphates (NTPs); thus, a lack of magnesium disrupts cell operations that require energy (Wendel et al. 2022). Since cells require magnesium to produce energy, a reduction in magnesium content in *Zymomonas mobilis* led to reduced cell growth (Li et al. 2020). The growth of *Burkholderia gluma*, however, was adversely affected by the presence of magnesium in the culture media (Nickzad et al. 2018).

However, the growth of UBCF_13 was not significantly affected by NaCl (Figure 1), in contrast to some bacteria, such as *Pseudomonas* sp. GO2, which required it to promote cell development (Feng et al. 2022). The elimination of NaCl and $K_2HPO_4$ in yeast sucrose medium did not produce a reduction in the IAA production of UBCF_13 (Figure 2). This outcome demonstrated that NaCl and $K_2HPO_4$ did not contribute significantly to IAA production. In *Bacillus* sp. strain MRN16, IAA production was optimized by the addition of potassium (Baliyan et al. 2021). In contrast, NaCl was found to be the main inorganic salt that promoted IAA production by *Pseudarthrobacter* sp. NIBRBAC000502770, *Providencia* sp., and rhizosphere bacteria from *Acacia cyanophylla* (Lebrazi et al. 2020; Ham et al. 2021; Fan et al. 2023). The production of IAA by *Bacillus subtilis* CW-2 was also increased by the addition of 0.5% and 1% NaCl to the culture medium (Yousef 2018).

### *3.5. Application of several indole compounds as precursor*

Different indole compounds were then added to the novel culture medium (yeast sucrose with $MgSO_4$ and $CaCO_3$) as precursors to confirm the IAA biosynthesis pathway in UBCF_13. The highest IAA production was found in the medium with TRP addition based on HPLC analysis (Figure 4). It is the most important precursor for IAA synthesis in bacteria (Figueredo et al. 2023). Tryptophan is known as the main substrate for IAA synthesis and can be converted into several intermediates (IAM, IPyA, TAM, IAN), which are grouped as tryptophan-dependent pathways (Zhang et al. 2019; Lin et al. 2022). In the IAM pathway, tryptophan is first converted into IAM by tryptophan 2-monooxygenase, followed by the conversion of IAM to IAA by hydrolase or amidase enzyme (Lin et al. 2022). The presence of the IAM compound in the medium with the addition of several indole compounds supported the use of the IAM pathway in UBCF_13 (Figure 3). This result was consistent with earlier studies identifying IAM as the sole intermediate detected in UBCF_13 extract (Yusfi et al. 2022, 2023a). An IAM peak was also detected in the medium with the addition of IAN (Figure S1D) because IAN could be converted into IAM via nitrile hydratase (Zhao et al. 2020). qRT-PCR analysis from a previous study also revealed that nitrile hydratase encoding genes, *nthA* and *nthB* were upregulated in response to several optimization treatments for IAA production. These results therefore confirmed that UBCF_13 used the IAM pathway to synthesize IAA. Indole compound was added to the culture medium to synthesize IAA. Indole compound was added to the culture medium to verify the IAA biosynthesis pathway in microorganisms. IAN, TAM, IPyA and IAM were added to the culture medium of Rhodosporidiobolus fluvialis DMKU-CP293; however, IAA was only observed in the medium to which IPyA was added (Bunsangiam et al. 2019). Another study also verified the IAA biosynthesis pathway by adding IAN to the buffer containing bacteria. HPLC analysis detected IAM and IAA in the buffer with *Variovorax boronicumulans* CGMCC


(a)




(b)

**FIGURE 4** a. Effect of UBCF_13 culture on chili seedlings at 4 weeks old. Chili seed treated with: aquadest for 30 mins (1); aquadest for 45 mins (2); aquadest for 60 mins (3); UBCF_13 culture for 30 mins (4); UBCF_13 culture for 45 mins (5); UBCF_13 culture for 30 mins (6); 1:2 dilution of UBCF_13 culture for 30 mins (7); 1:2 dilution of UBCF_13 culture for 45 mins (8); 1:2 dilution of UBCF_13 culture for 60 mins (9); 1:4 dilution of UBCF_13 culture for 30 mins (10); 1:4 dilution of UBCF_13 culture for 45 mins (11); 1:4 dilution of UBCF_13 culture for 60 mins (12); UBCF_13 culture supernatant for 30 mins (13); UBCF_13 culture supernatant for 45 mins (14); UBCF_13 culture supernatant for 60 mins (15); 1:2 dilution of supernatant for 30 mins (16); 1:2 dilution of supernatant for 45 mins (17); 1:2 dilution of supernatant for 60 mins (18); 1:4 dilution of supernatant for 30 mins (19); 1:4 dilution of supernatant for 45 mins (20); 1:4 dilution of supernatant for 60 mins (21). b. Effect of UBCF_13 culture and supernatant under different soaking durations on root length (a), shoot length (b), fresh weight (c), and germination time (d) of chili seedlings at 4 weeks old. Aq= aquadest; C= UBCF_13 culture; C (1:2)= 1:2 dilution of UBCF_13 culture; C (1:4)= 1:4 dilution of UBCF_13 culture; S= supernatant of UBCF_13 culture; S (1:2)= 1:2 dilution of supernatant; S (1:4)= 1:4 dilution of supernatant. Values were the means of three replicates ±SD. Lowercase letters represent significant differences among treatments by DMRT (alpha = 0.05%).

4969 cells and IAN addition (Sun et al. 2018). However, a similar experiment found no IAA in the buffer containing *Ensifer meliloti* CGMCC 7333 with the addition of IAN (Zhao et al. 2020).

### 3.6. Application of UBCF_13 extract to chili

UBCF_13 culture and supernatant of UBCF_13 cultivated on the novel culture medium were also used to improve the germination of chili seeds. UBCF_13 culture showed a negative effect on the growth of chili seedlings (Figure 4a). This contrasted with Patel et al. (2022), who found that chili growth was significantly increased by IAA-producing *Curtobacterium oceanosedimentum* DG-20 inoculation. The plant growth-promoting bacteria cells may have a beneficial or deleterious effect when inoculated into the seeds. The formation of bacterial biofilm on the seed surface and its detrimental effect on plant-bacterial interaction could inhibit the seed germination process (Tiwari and Singh 2017; Lobo et al. 2023). In contrast to the UBCF_13 culture, the supernatant significantly enhanced shoot length, root length, and fresh weight, in addition to triggering seed germination in chili (Figure 4b). This result aligned with the previous study by Aisyah et al. (2019), which found that UBCF_13 supernatant cultivated on LB medium enhanced the root and shoot length of *Solanaceous* seeds. At that time, however, the effect of soaking time and the dilution of bacterial supernatant had not previously been widely studied. Studies on the effect of the inoculation of plant growth-promoting bacteria on plant seeds used soaking times ranging from 30 mins to 24 h (Sunera et al. 2020; Shah et al. 2020; Liu et al. 2020; Fahsi et al. 2021; Kaur and Manhas 2022). In this study, different soaking durations (30 mins, 45 mins, and 60 mins) did not influence the growth and development of chili seeds, except for the shoot length (Figure 4b). The soaking time of the seeds might need further observation in a shorter or longer time to give a significant result. Another study showed that the soaking time of tomato seeds with PGPR culture for 10 and 20 mins significantly increased the percentage of seed germination (Widnyana and Javandira 2016). Meanwhile, the soaking time of rice seeds with *Streptomyces* culture for 12 h showed a higher positive impact on the growth of rice seedlings compared to 6 h (Hata et al. 2021).

The UBCF_13 culture supernatant with 1:2 and 1:4 dilution showed the same effect on seed growth (except for shoot length and fresh weight) as the absolute (no dilution) supernatant (Figure 4b). The IAA content of the supernatant with 1:2 and 1:4 dilution was 69.4 and 35.2 µg/mL, respectively (Figure 5). Hence, it can be inferred that seed germination required the addition of IAA at a low concentration (below 100 µg/mL). Low IAA concentration also increased the growth of *Agastache rugosa* and the seed yield of *Guizotia abyssinica* (L.f.) Cass (Lam et al. 2020; Talukdar et al. 2022). The high level of IAA can trigger the enzyme aminocyclopropane-1-carboxylic acid synthase (ACC synthase) and inhibit the rate of cyclosis. These activities lead to excessive ethylene and toxin production that disturb plant growth (Bunsangiam et al. 2021; Talukdar et al. 2022).

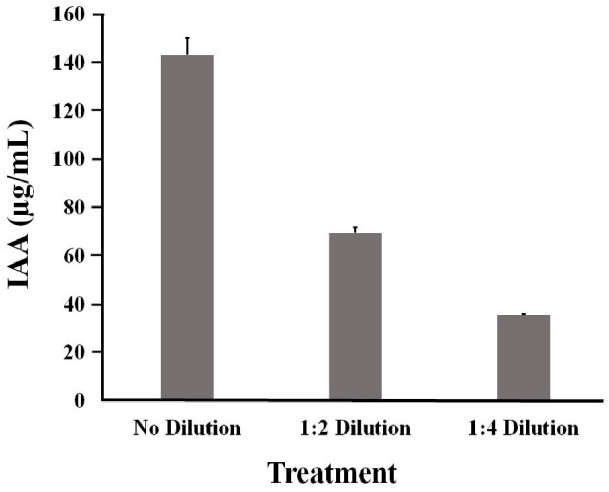



**FIGURE 5** IAA content from dilution of supernatant of UBCF_13 culture. Values are the means of three replicates ±SD.

## 4. Conclusions

This study found that the elimination of $K_2HPO_4$ and NaCl in yeast sucrose medium had minimum effect in the decrease of bacterial density and IAA production by UBCF_13. However, the IAA production decreased significantly when $MgSO_4$ and $CaCO_3$ were removed. Therefore, the new composition of culture medium for IAA production by UBCF_13 consists of sucrose, yeast extract, $MgSO_4$, and $CaCO_3$ with the addition of 300 µg/mL tryptophan. The addition of several indole compounds as precursors confirmed that the IAM pathway was used to synthesize IAA in UBCF_13. The application of UBCF_13 culture supernatant at a 1:4 dilution significantly enhanced the germination and early development of chili. Therefore, the UBCF_13 extract grown in this medium offers the potential for use as a biofertilizer.

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## Authors' contributions

LAY and JJ planned the experiment. LAY conducted the experiments, statistically analyzed the data and wrote the original draft. DHT and IC contributed as research supervisor and reviewed articles. MI executed the SDS-PAGE and plant assay. JJ contributed as research supervisor, reviewed manuscript, and funding acquisition. All

authors contributed and approved the final editing of the manuscript.

## Competing interests

All authors declare no conflict of interest.

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