Culture medium optimization for Indole-3-Acetic Acid production by Serratia plymuthica UBCF_13

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Abstract. Serratia plymuthica UBCF_13 is one of the bacteria that can increase plant growth [plant growth-promoting bacteria] by producing IAA [Indole-3-Acetic Acid]. S. plymuthica UBCF_13 is a strain of Andalas University Biotechnology Laboratory Collection which can produce IAA and increase the growth of Solanaceae plants. Optimization of culture media needs to be analyzed to increase IAA production on UBCF_13. Optimization can be done by adding tryptophan as a precursor, using various types of media, adding wall affecting agents, and certain metal ions. In this study, optimization was carried out by testing the type of media [TSB, NB, YM, and King’s B], adding tryptophan [0, 100, 200, 300 μg/mL], differences in pH [5, 6, 7, 8], giving wall affecting agent [SDS 0.1 μg/mL, EDTA 0.1 μg/mL], and metal ions [Ca, Fe, K, Mg at a concentration of 0.05% and 0.1%]. The highest IAA production was obtained in the combination treatment of YM media and tryptophan 300 μg/mL. Meanwhile, the treatment of differences in pH and wall affecting agents did not have a significant effect on the increase in the production of IAA UBCF_13. Testing of metal types on IAA production showed that calcium was able to increase the production of IAA UBCF_13 by 12-14 μg/mL. Serratia plymuthica UBCF_13 produced the highest IAA on YM media combined with the addition of 300 μg/mL of tryptophan and 0.1% calcium ion.

Keywords: IAA production, metals plant growth-promoting bacteria, serratia, wall affecting agents

1. Introduction

Plant Growth Promotions Bacteria [PGPB] belong to bacteria that can stimulate plant growth through various roles, one of which is by producing the phytohormone Indole-3-Acetic Acid [IAA] [1]. IAA is the most well-known auxin and can influence the growth of plant cells [2]. Therefore, PGPB can also be utilized to produce commercial IAA. Commercial IAA is needed in plant tissue culture as a regulator for organ formation, callus induction, and somatic embryogenesis induction [3]. One of the PGPB strains known to be able to produce IAA is Serratia plymuthica UBCF_13.

Aisyah et al. [4] reported that the UBCF_13 strain cultured on LB media for 48 hours with the addition of 0.2% tryptophan was able to produce IAA of 116.09 μg/mL. IAA produced from UBCF_13 cells-free supernatants [CFS] could enhance the elongation of root and shoot of Solanaceae plants. However, the production of IAA UBCF_13 at various pH levels was carried out by Wandira et
al. [5] decreased by 79%. Therefore, the increase in IAA UBCF_13 production needs to be continued
by using commercial media. Kalimuthu et al. [6] and Bhutani et al. [7] have been optimized the IAA
production of bacteria using commercial media. IAA production of Pseudomonas sp. VSMKU4050
was found highest in King’s B medium. However, Bacillus spp. produced the highest IAA production
on YEM media with 500 μg/mL tryptophan. Wagi and Ahmed [8] also added EDTA as a wall
affecting agent [WAA] which showed that the production of IAA B. cereus [So3II] and B. subtilis
[Mt3b] increased by 88.3% and 94.6%. Another study reported that the addition of certain metal ions
also increased IAA production [9]. Hence, we optimized the production of IAA Serratia plymuthica
UBCF_13 using various commercial media, testing the best tryptophan concentration on the
commercial media, adding WAA, and giving metal ions.

2. Materials and methods

2.1. Preparation of Isolates and Culture Media
S. plymuthica UBCF_13 was acquired from FP-UA Biotechnology Laboratory Collection and was
cultured on LB [Luria Bertani]. All treatments used complete block design. Each treatment was
incubated for 48 hours in a shaking incubator [160 rpm, 28 °C].

2.2. Optimization with Different Types of Media and Concentrations of Tryptophan
Four commercial media [Yeast Extract Mannitol [YEM] Broth, King’s B, Nutrient Broth [NB], and
Tryptone Soybean Broth [TSB] [HIMedia] were used as media culture. Optimization of IAA
production in each medium was also carried out by adding tryptophan at various concentrations [0,
100, 200, 300 μg/mL] regarding testing by Bhutani et al. modified [7].

2.3. Optimization with Different pH and Addition of WAA
UBCF_13 then was cultured at various pH conditions [5, 6, 7, 8] and combined with the addition of
WAA [0.1% SDS, 0.1% EDTA] based on Wagi and Ahmed [8].

2.4. Optimization with the Addition of Metal Ions
The next step is metal ions addition [FeSO4, MgSO4, CaCl2, KNO3 [0.05% and 0.1%]] following the
procedure of Aisyah et al. with modifications[4].

2.5. Measurement of Bacterial Density
The bacterial density was measured using a spectrophotometer at 600 nm. The absorbance value is
converted into cells per mL.

2.6. IAA Production Screening
IAA production in each treatment was measured using a colorimetric method. IAA screening for each
sample requires a regression line equation obtained from a standard curve. The IAA measurement
process for each sample was started by centrifuging the bacterial culture media for 10 minutes at
10,000 rpm. Furthermore, 2 mL of supernatant was taken and dissolved with 4 mL of Salkowski
reagent. The solution was incubated for 30 minutes in dark conditions then the wavelength of 530 nm
was used to measure the absorbance. Measurements for each sample were repeated three times.

2.7. Statistical analysis
IAA production data for each treatment were analyzed using two-way ANOVA through SPSS version
24.0 software. Further tests were carried out with the Duncan Multiple Range Test [significance =
0.05].
3. Results and discussion

3.1. Optimization with Different Types of Media and Concentrations of Tryptophan

The bacterial density in YM media increased with 100 µg/mL of tryptophan \([1.5 \times 10^9 \text{ cells/mL}]\) meanwhile in King's B media without the addition of tryptophan was lower \([1.5 \times 10^9 \text{ cells/mL}]\) than other treatments. Conversely, NB media obtained the same cell density in all treatments \([1.5 \times 10^9 \text{ cells/mL}]\). TSB media was the best medium for the growth of UBCF_13 [Figure 1]. This is supported by a higher bacterial density than other media \([1.8 \times 10^9 \text{ cells/mL}]\). Vijayakumari et al. also found that TSB media produced higher bacterial growth than NB and LB media[10].

![Figure 1. UBCF_13 bacterial density based on differences in media types and tryptophan concentrations. Note: A1 = YM media; A2 = Media King's B; A3 = NB media; and A4 = TSB media. B1 = tryptophan 0 µg/mL; B2 = tryptophan 100 µg/mL; B3 = tryptophan 200 µg/mL and B4 = tryptophan 300 µg/mL.](image-url)

The IAA production data on TSB media tends to be higher than treatment on other media except for the addition of 300 µg/mL tryptophan [Figure 2]. On the other hand, IAA cannot be produced in King's B media with the addition of 0 - 300 µg/mL of tryptophan. Production of IAA without the addition of tryptophan [B1] on YM and NB media also produced ± 0 µg/mL of IAA. IAA production in the addition of tryptophan in NB media was lower than YM and TSB media. However, the highest IAA production \([± 95 \mu g/mL]\) was obtained from the combination of YM media with 300 µg/mL of tryptophan. YM media is generally used in the production of IAA by PGPR of the Rhizobium genus such as Rhizobium species isolated from *Cynamopsis tetragonoloba* [L.], Rhizobium isolates isolated from peanuts, Rhizobium tropici CIAT 899 [11–13]. The production of IAA is also significantly increased based on the concentration of tryptophan added to the culture media. Tryptophan is an essential amino acid with a nitrogen content of 14% and serves as a precursor in the synthesis of IAA [14]. Previously Aisyah et al. have obtained that 0.2% was the optimal concentration of tryptophan in IAA production by *Serratia plymuthica* UBCF_13. The optimum tryptophan concentration in this study was higher than the optimum tryptophan concentration in the study of Aisyah et al. [4]. This study shows that the amount of optimum tryptophan concentration to produce IAA depended on the different compositions of the media used.
Figure 2. Production of IAA UBCF_13 based on differences in media types and tryptophan concentrations. Note: A1 = YM media; A2 = Media King's B; A3 = NB media; and A4 = TSB media. B1 = tryptophan 0 µg/mL; B2 = tryptophan 100 µg/mL; B3 = tryptophan 200 µg/mL and B4 = tryptophan 300 µg/mL.

3.2. Optimization with Different pH and Addition of WAA
The bacterial density in cultures with different pH and WAA ranged from $1.18 \times 10^9$ to $1.3 \times 10^9$ cells/mL [Figure 3]. The bacterial culture at pH 5, 6, 7, 8 with and without the addition of WAA in YM media did not produce a significant difference in bacterial density. Wandira et al. [unpublished] also have studied the effect of pH and the highest IAA production was found at pH 6. Another study conducted by Rushabh et al. showed that the highest bacterial density values were in treatment with pH 8 and were not significantly different at pH 7, 7.5, and 9 [15].

Figure 3. Bacterial Density based on pH differences and the addition of wall affecting agents. Note: W0 = No wall affecting agent; WS = SDS 0.1 µg/mL; WE = EDTA 0.1 µg/mL.
IAA production in cultures with differences in pH and the addition of WAA was not statistically different [Figure 4]. IAA Production with EDTA 0.1 µg/mL at pH 5 [100.62 µg/mL] was higher than other treatment. In contrast, IAA production with EDTA 0.1 µg/mL [96.69 µg/mL] was lower than the other two treatments on media at pH 6. Production of IAA in the treatment without WAA at pH 7 [95.51 µg/mL] was lower than the treatment with WAA [102 µg/mL]. Cultures without WAA at pH 8 produced higher IAA [98.65 µg/mL] than cultures with WAA. Other studies have shown that the pH value of the media for IAA production by PGPB varies. IAA production of UBCF_13 that has been analyzed in LB medium by Wandira et al. [5] showed the optimum pH was 6. *Serratia marcescens* subsp. *marcescens* strain KB01 and *Serratia marcescens* subsp. *marcescens* strain KB05 produces IAA optimally at pH 9 while in *Providencia* sp. strain 7MM11 was obtained at pH 8 [15,16]. Related to the addition of WAA previously tested by Wagi and Ahmed in the genus *Bacillus*, IAA production increased by 88.3% - 94.6% [8]. EDTA in this case acts as a cell wall softening agent in bacteria so that the release of IAA from inside bacterial cells can be increased.

![Figure 4](image.png)

**Figure 4.** Production of IAA based on pH differences and the addition of wall affecting agents. Note: WS = SDS 0.1 µg/mL; WE = EDTA 0.1 µg/mL.

### 3.3. Optimization with the Addition of Metal Ions

Measurement of bacterial density in treatment with the addition of metal ions showed the highest bacterial density at the addition of 0.05% Mg ions [1.48 x 10⁹ cells/mL] [Figure 5]. The bacterial density with the addition of 0.1% K ion was higher than the density of bacteria without metal addition. Imada et al. also tested the effect of potassium ions on bacterial growth and the results showed that there was a significant increase in the growth of the bacteria *Rhizobium tropici* CIAT 899 [13]. The treatment with the addition of Ca, Fe, K 0.05%, and 0.1% Mg resulted in lower bacterial density compared to the no treatment sample. The addition of metal ions to bacteria can be toxic if the amount is excessive so that it may reduce the density of the bacteria [17].
Figure 5. Optical density is based on differences in metal ions.

On the other hand, the bacterial density value in culture with Ca ions produced lower values than other samples, the IAA production in this treatment resulted in the highest value compared to the addition of other metal ions. The addition of Ca ions showed an increase in IAA production by 12-14 µg/mL compared to the no treatment [Figure 6]. IAA is a secondary metabolite product where its production can be affected by the addition of metal ions. Several gene groups that are responsible for the formation of secondary metabolites could be activated by adding metal ions to increase the production of secondary metabolites [18]. In Fe addition, IAA production was 50% lower than without treatment. This indicates that the Fe ion can inhibit the production of IAA UBCF_13. A study related to the effect of metals on IAA production was also carried out by Jalali et al. using Cd, Sr, Ce, La, Nd, and Y showed that there were strains [G1B1, G1B2, M1B4, M1B5, M7B2, S3B1, and P. aeruginosa] which had increased IAA production on the addition of metal ions. On the other hand, strain G1B3 and P. fluorescens were not significantly affected by the presence or absence of metals, and strain G3B2 was inhibited in producing IAA by the addition of metal [19].

Figure 6. IAA Production based on differences in metal ions.
4. Conclusion
Serratia plymuthica UBCF_13 produced the highest IAA on YM media combined with the addition of 300 µg/mL of tryptophan and 0.1% calcium ion.

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