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Short Communication

The effect of tryptamine on *Serratia marcescens*, *Pseudomonas aeruginosa* and *Escherichia coli*

Arif Luqman[1,2]*, Ruben Amian Ruiz[2], Friedrich Götz[2], Maya Shovitri[1], Enny Zulaika[1], Dewi Hidayati[1] and Triono Bagus Saputro[1]

[1]Biology Department, Institut Teknologi Sepuluh Nopember, 60111 Surabaya, Indonesia.
[2]Microbial Genetics, University of Tuebingen, 72076 Tuebingen, Germany.
Email: arif.luqman@its.ac.id

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ABSTRACT

Aims: Tryptamine is an amine compound derived from tryptophan by decarboxylation process. This compound can be found in fermented food and beverages, and in human gut and skin as well. This study aims to investigate the effect of tryptamine, on Gram-negative bacteria, namely *Escherichia coli*, *Serratia marcescens* and *Pseudomonas aeruginosa*.
Methodology and results: In this study, we used *E. coli*, *S. marcescens* and *P. aeruginosa* due to their relatively observable quorum sensing-regulated phenotype, such as motility, prodigiosin and pyocyanin sequentially. Our results showed that tryptamine started to inhibit the growth and prodigiosin production of *S. marcescens* at concentration 250 µg/mL, while it inhibits the growth and pyocyanin production of *P. aeruginosa* at concentration 250 µg/mL and 500 µg/mL, respectively. Tryptamine inhibits both the growth and motility of *E. coli* at concentration 100 µg/mL.
Conclusion, significance and impact of study: These results suggest that tryptamine is able to inhibit the growth of *E. coli*, *S. marcescens* and *P. aeruginosa* at relatively high concentration, thus decreases the quorum sensing-regulated phenotypes. It implies that the growth and quorum sensing of Gram-negative bacteria most likely will not be affected by the low concentration of tryptamine that present in the gut.

Keywords: Tryptamine, Gram-negative bacteria, growth, quorum sensing-regulated phenotype

INTRODUCTION

Tryptamine is an amine compound produced from a decarboxylation process of an aromatic amino acid, tryptophan. Tryptamine can be produced by many organisms such as mammals, human, plants, bacteria and fungi (Halász *et al*., 1994; Mercolini, 2019). Tryptamine has a broad potential due to its capability to interact with various receptors found in mammals, such as α1- , α2- and β-adrenergic receptor, serotonin 1A and 2A receptor ($5HT_{1A}$ and $5HT_{2A}$), and trace amines-associated receptor 1 (TAAR1) (Greene, 2013; Berry *et al*., 2017; Luqman *et al*., 2018, 2019, 2020a, 2020b) . In human, tryptamine can be found in brain, gut and the bacteria which inhabited in the gut as well (Berry, 2004; Luqman *et al*., 2019). It was reported that this particular compound able to induce the intestine motility (Williams *et al*., 2014) and increase bacterial adherence and internalization into intestine (Luqman *et al*., 2018). However, the presence of high concentration tryptamine might lead to gastrointestinal disorder due to the over expression of TAAR1 in the gut (Gwilt *et al*., 2020).

Human intestine is an organ where microorganisms inhabit with the most number compared to the other organs. As tryptamine can be found in intestine, it is possible that tryptamine might also affect the microorganisms there. Although it is reported that tryptamine showed no significant effect on bacterial adrenergic receptor QseC, a Gram-negative quorum sensing which can detect adrenaline (Luqman *et al*., 2020a), we wonder whether other quorum sensing systems in Gram-negative bacteria can be affected. Here, we showed that tryptamine interferes with the growth of Gram-negative bacteria, namely *Serratia marcescens*, *Pseudomonas aeruginosa* and *Escherichia coli* at relatively high concentration (≥ 100 µg/mL).

***Corresponding author**

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MATERIALS AND METHODS

Bacterial strains and growth conditions

In this study, 3 different bacterial strains: *E. coli* K12*, S. marcescens* DSM48 and *P. aeruginosa* PAO1 were used. All of the bacterial strains are obtained from the collection of Microbial Genetics Department, University of Tuebingen. Bacteria were cultivated in basic medium (BM) (1% soy peptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose and 0.1% K_2HPO_4, pH 7.2) at 37 °C with continuous shaking at 150 rpm for 24 h.

Growth inhibition tests

The growth inhibition test was carried out in 96 well plates. *Serratia marcescens* and *P. aeruginosa* were inoculated separately and incubated overnight as preculture. The preculture was then reinoculated into fresh BM medium with OD_{600} 0.01 and added to the wells with final volume of 150 µL per well. Tryptamine (Merck) was then added into the wells at concentrations of 15, 30, 62, 125, 250, 500 and 1000 µg/mL. The cultures were then incubated at 37 °C and continuous shaking at 150 rpm and after 24 h, the OD_{600} was measured with plate reader (Tecan, Germany).

Prodigiosin production assay

Serratia marcescens was inoculated into a fresh liquid BM and incubated overnight at 30 °C with 150 rpm agitation. The bacterial culture from overnight culture was then reinoculated into fresh liquid BM and adjusted to OD_{600} of 0.001 at volume 200 µL in 96 well plates. The bacteria were then treated with different concentrations (0, 15, 30, 62, 125, 250, 500 and 1000 µg/mL) of tryptamine and incubated for 24 h at 30 °C as *S. marcescens* stops producing prodigiosin if the temperature higher than 30 °C (Elkenawy *et al*., 2017). After 24 h, the cell density was measured at 600 nm. Then, the bacterial cultures were then pelleted and the red pigment prodigiosin were extracted using acidified ethanol modification from Thomson *et al.* (2000). The relative prodigiosin production was calculated as the ratio between the amount of prodigiosin and violacein at its maximum absorption at 534 nm for prodigiosin, and cell density values at 600 nm.

Pyocyanin production assay

Pseudomonas aeruginosa was inoculated into a fresh liquid BM and incubated overnight at 30 °C with 150 rpm agitation. The bacterial culture from overnight culture was then reinoculated into fresh liquid BM and adjusted to OD_{600} of 0.001 at volume 200 µL in 96 well plates. The bacteria were then treated with different concentrations (0, 15, 30, 62, 125, 250, 500 and 1000 µg/mL) of tryptamine and incubated for 24 h at 30 °C. After that, the cell density was measured at 600 nm using plate reader (Tecan, Germany), while the supernatants were subjected to HPLC (Agilent technologies, Waldbronn, Germany) separation by using Nucleosil 100, C-18 column (Macherey Nagel, Düren, Germany) with a 15 min linear gradient of 0.1% phosphoric acid to acetonitrile at a flow rate of 1.5 mL/min to measure the pyocyanin production (Chu *et al*., 2013). The concentration of pyocyanin was determined at its maximum absorption at 520 nm using diode-array detection (DAD) detector. The relative pyocyanin production was calculated as the ratio between the measured pyocyanin using HPLC and cell density values at 600 nm using plate reader.

Motility assay

The motility of *E. coli* was measured using a standard motility asay (Croze *et al*., 2011). *E. coli* was inoculated in BM and incubated overnight at 37 °C with continuous shaking at 150 rpm and used as pre-culture. The pre-cultures were then re-inoculated into fresh BM medium and adjusted to get OD_{600} of 0.1 and incubated for 4 h to reach mid-log phase. The culture was taken 3 µL and dropped on freshly prepared motility agar medium (1% tryptone, 0.5% yeast extract, 1% NaCl and 0.25% agar) containing different concentrations of tryptamine (0, 25, 50 and 100 µg/mL), respectively. The plates were then incubated at 37 °C. At 24 h, the radius of swarming area was measured by measuring the radius at 3 different positions and calculate the mean.

Growth curve

Escherichia coli was inoculated in BM medium and incubated overnight and used as pre-culture. The pre-cultures were then re-inoculated in fresh BM medium at initial OD_{600} of 0.1. The OD_{600} were then measured every 2 h for 24 h.

RESULTS

High concentrations of tryptamine inhibit the growth of *S. marcescens* and prodigiosin production

The aim of this study was to investigate the effects of tryptamine on the growth and quorum sensing (QS) of Gram-negative bacteria. In this study, *S. marcescens* was chosen due to its known QS-regulated pigment production, prodigiosin (Thomson *et al*., 2000). Based on Figure 1, it shows that starting from concentration 125 µg/mL, tryptamine inhibits significantly both the growth and the prodigiosin production. Meanwhile, at 62 µg/mL and lower concentrations, there was no significant effect observed.

High concentrations of tryptamine inhibit the growth of *P. aeruginosa* and pyocyanin production

Similar to *S. marcescens*, pyocyanin production is regulated by quorum sensing in *P. aeruginosa* (Dietrich *et al*., 2006). The assays showed that tryptamine at the concentration of 250 µg/mL can significantly inhibit the growth of *P. aeruginosa*. Moreover, at the concentration

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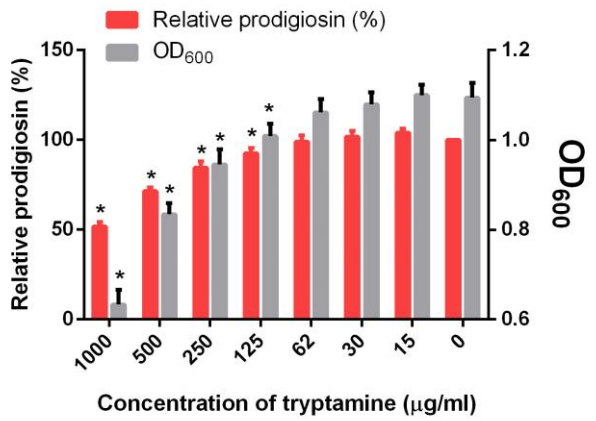

Figure 1: High concentrations of tryptamine inhibit the growth of *S. marcescens* and prodigiosin production. Each data point in this graph represents the mean value ± SD from 3 independent replications, *$p < 0.05$. The statistical analysis used was ANNOVA test.

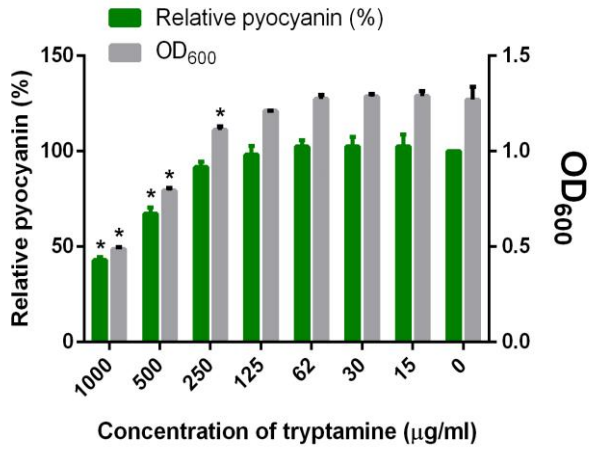

Figure 2: High concentrations of tryptamine inhibit the growth of *P. aeruginosa* and pyocyanin production. Each data point in this graph represents the mean value ± SD from 3 independent replications, *$p < 0.05$. The statistical analysis used was ANNOVA test.

of 500 µg/mL, tryptamine starts to inhibit the relative pyocyanin production (Figure 2).

Tryptamine inhibits the motility and growth of *E. coli*

As motility is one of the QS-regulated phenotypes in *E. coli* (Sperandio *et al*., 2002), the effect of tryptamine on *E. coli* motility was investigated by dropping the mid-log phase of *E. coli* culture on motility agar and measured the radius of swarming area after 24 h incubation. The radius of swarming area was reduced significantly in the presence of tryptamine at concentration of 100 µg/mL compared to control (0 µg/mL of tryptamine) (Figure 3A). Similar results were also observed for the growth, 100 µg/mL tryptamine inhibit the growth of *E. coli* significantly (Figure 3B).

DISCUSSION

In recent study, the interaction between trace amine and QseC, a quorum sensing receptor that is found on some Gram-negative bacteria has showed that tryptamine has no effect on QseC histidin kinase activity (Luqman *et al*., 2020a). The possible interactions or effects of tryptamine and other quorum sensing receptors in Gram-negative bacteria are not yet ruled out. Hence, the effects of tryptamine on some Gram-negative bacteria were investigated in this study.

The effects of tryptamine have been tested on *S. marcescens*, *P. aeruginosa* and *E. coli* as these species have quorum sensing-regulated-phenotype which can be observed using relatively simple experiments. The results showed that tryptamine decreased the quorum sensing-regulated phenotype such as prodigiosin production in *S. marcescens*, pyocyanin production in *P. aeruginosa* and motility in *E. coli* at high concentration (≥ 100 µg/mL). Besides, the tryptamine's interference in their growth was also observed. At high concentration, tryptamine inhibited the growth of these bacteria at similar or even lower concentration as the quorum sensing-regulated phenotype was shown to be decreased. These results suggested that tryptamine decreases the quorum sensing-regulated phenotype not due to its interfererence with quorum sensing system but due to its inhibition on the growth of the bacteria.

In this study, the quorum sensing-regulated phenotypes, such as prodigiosin and pyocyanin production and motility were observed to be significantly decreased in the presence of relatively high concentration of tryptamine. At similar concentration, tryptamine showed to inhibit the bacterial growth significantly. It was reported that the highest concentration of tryptamine detected in fecal samples is around 60 µg/g feces (Luqman *et al*., 2019), which is much lower concentration than the concentration where it showed a significant effect on bacterial growth. Therefore, the decrease of prodigiosin in *S. marcescens*, pyocyanin in *P. aeruginosa* and motility in *E. coli*, which are quorum sensing-regulated phenotypes (Thomson *et al*., 2000; Sperandio *et al*., 2002; Dietrich *et al*., 2006), were most probably due to the low density of bacterial cells as tryptamine inhibited the bacterial growth.

The mechanism of how tryptamine affects the growth of Gram-negative bacteria remains unknown. It deserves a separate research project as deeper understanding on how tryptamine works and more sophisticated experiments to elucidate. For example, predicting the possible interaction or tryptamine with some proteins using *in silico* analysis and comfirm it with *in vitro* experiments by purifying the candidate proteins and measuring the interaction using surface plasmon experiment and further investigate the effect of the interaction with *in vivo* experiment by constructing mutants of certain candidate genes or supplementation with certain compound that can

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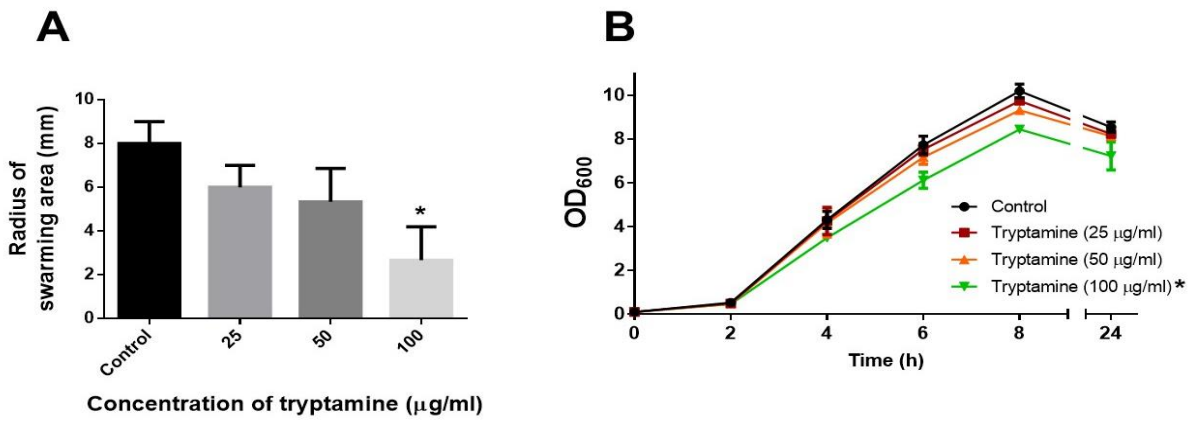

Figure 3: Tryptamine inhibits the (A) motility and (B) growth of *E. coli*. Each data point in these graphs represents the mean value ± SD from 3 independent replications. *p < 0.05. The statistical analysis used was unpaired Student's t test for graph (A) and paired Student's t test for graph (B).

antagonize the effect of tryptamine.

CONCLUSION

In this study, the effects of tryptamine on *S. marcescens*, *P. aeruginosa,* and *E. coli* which represent Gram-negative bacteria was investigated. The results showed that tryptamine inhibit the growth of the Gram-negative bacteria at relatively high concentration, thus decrease the quorum sensing-regulated phenotype following effect of growth inhibition.

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