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The effects of dopamine at physiological concentration on the growth and biofilm formation of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*

Original Article

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Abstract:

Iron is an essential macronutrient for almost all living organisms, including pathogenic bacteria. Pathogenic bacteria have developed iron acquisition mechanisms due to the low bioavailability of iron inside the host. One of them is ability to utilize dopamine as a xenosiderophore. In this study we investigated the effects of dopamine at physiological concentrations (0.1–2 μM) on the growth and biofilm formation of bacteria grown in iron-limited and iron-enriched media. The significant growth enhancement was observed in *Staphylococcus aureus* USA300 grown in iron-enriched media. Dopamine at the concentration of 1 μM increased biofilm formation in *S. aureus* USA300, *Staphylococcus epidermidis* O47, and *Escherichia coli* K12 in both iron-limited and iron-enriched media after 3 days of incubation. These findings provide an insight into the effects of dopamine on bacteria at the physiological concentrations.

Key words:
dopamine, siderophore, biofilm, bacteria, microorganisms

Apstrakt:

Efekat dopamina u fiziološkoj koncentraciji na rast i formiranje biofilma kod vrsta *Staphylococcus aureus*, *Staphylococcus epidermidis* i *Escherichia coli*

Gvožđe je osnovni makronutrijent za gotovo sve žive organizme, uključujući i patogene bakterije. Zbog niske bioraspoloživosti gvožđa unutar domaćina, patogene bakterije su razvile mehanizme za njegovo usvajanje. Jedan od tih mehanizama je sposobnost korišćenja dopamina kao ksenosiderofora. U ovoj studiji ispitivali smo uticaj dopamina u fiziološkim koncentracijama (0,1-2 μM) na rast i formiranje biofilma kod bakterija gajenih na podlogama sa ograničenim i obogaćenim sadržajem gvožđa. Značajno poboljšanje rasta uočeno je kod soja *Staphylococcus aureus* USA300 gajenog u podlozi sa obogaćenim sadržajem gvožđa. Dopamin u koncentraciji od 1 μM povećao je formiranje biofilma kod sojeva *S. aureus* USA300, *Staphylococcus epidermidis* O47 i *Escherichia coli* K12, i u uslovima sa ograničenim i obogaćenim sadržajem gvožđa, nakon tri dana inkubacije. Ovi nalazi pružaju uvid u delovanje dopamina na bakterije u fiziološkim koncentracijama.

Ključne reči:
dopamin, siderofor, biofilm, bakterije, mikroorganizmi

Introduction

Iron (Fe) is an essential element for almost all living organisms, including pathogenic bacteria. Iron plays crucial roles in various processes, such as in cellular respiration (Blake et al., 1993; Levi & Rovida, 2009), DNA synthesis (Puig et al., 2017), as well as in several redox enzymes activities (Vigani & Murgia, 2018). Although iron is abundant in the environment, the bioavailability of iron inside the host is very limited due to the most of the iron is in complex form with proteins, such as hemoglobin, transferrin, and ferritin (Krzyminiewski et al., 2011), which makes it difficult for bacteria to access (Bullen, 1981). In order to overcome this limitation, bacteria have developed an iron acquisition mechanism through siderophore production by chelating iron from the environment thus making iron accessible by the bacteria through specific transport protein (Ellermann & Arthur, 2017; Kramer et al., 2020).

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Staphylococcus aureus, Staphylococcus epidermidis, and *Escherichia coli* are commensal bacteria that are naturally present in the human body (Escobar-Paramo et al., 2004; Luqman et al., 2018; Luqman et al., 2020). Under normal conditions, these three bacteria play an important role in maintaining the balance of the host microbiota. However, when immunity decreases or environmental changes occur, these bacteria can transform into opportunistic pathogens that cause a variety of infections, including skin infections, urinary tract infections, bacteremia, and medical device-associated infections due to biofilms (Otto, 2009; Dey & Ray Chaudhuri, 2023).

Biofilms are bacterial communities embedded in an extracellular matrix, providing protection from the host immune system and antibiotic therapy (Otto, 2006; Hanke & Kielian, 2012; Le et al., 2018). Iron availability plays a key role in biofilm formation, with iron deficiency inhibiting this process (Greenberg & Banin, 2008; Avidan et al., 2010). To overcome iron deficiency, bacteria use siderophores to obtain iron from the environment. However, in addition to siderophores, several small molecules such as catecholamines, including dopamine, are also known to play a role in increasing iron availability to bacteria (Dichtl et al., 2019; Perraud et al., 2022). Dopamine is an important neurotransmitter found in human body fluids, and its catechol group allows dopamine to interact with iron ions, forming a dopamine-iron complex that can be used as an iron source by bacteria (Luqman, 2023).

This study aimed to investigate the effect of dopamine on iron binding, growth, and biofilm formation by *S. aureus, S. epidermidis*, and *E. coli* in iron-limited and iron-enriched media conditions. We used dopamine concentrations that mimic physiological levels in the human body (0.1–2 µM) to ensure the relevance of the results to the *in vivo* situation. Thus, the results of this study provide new insights into the interaction of dopamine with bacteria as well as its implications for bacterial colonization and biofilm formation in the human body.

Materials and Methods

Chrome Azurol S (CAS) Assay

The CAS assay was performed according to previous publications (Schwyn & Neilands, 1987; Murakami et al., 2021). We used CAS solution which containing Chrome azurol S (0.15 mM) (Sigma), hexadecyltrimethylammonium bromide (0.6 mM) (Sigma), and $FeCl_3$ (1.25 mM) (Sigma). The siderophore activity of dopamine was investigated by mixing the CAS solution with dopamine at ratio 1:1 and the absorbance was measured using microplate reader at a wavelength of 630 nm. The amount of iron bound was calculated by using a standard curve which was prepared prior the assay by measuring the absorbance of CAS solution containing various concentrations of $FeCl_3$.

Bacterial Growth Curve

Bacterial strains, *Staphylococcus aureus* USA300, *Staphylococcus epidermidis* O47, and *Escherichia coli* K12, were grown in tryptic soy broth (TSB) (Himedia) overnight at 37 ºC with agitation and used as preculture. The precultures were pelleted, washed, and resuspended in sterile phosphate buffer. The resuspended bacterial cells were then inoculated into MKB medium (2.5 g/L K_2HPO_4 (Sigma); 2.5 g/L $MgSO_4 \cdot 7H_2O$ (Sigma); 15 ml/L glycerin (Sigma); 5.0 g/L casamino acids (Himedia) and the pH was adjusted to 7.2) (Gu et al., 2021). The iron limited MKB medium was supplemented with 10 µM of $FeCl_3$ (Sigma) while the iron enriched MKB medium was supplemented with 50 µM of $FeCl_3$. Dopamine was added to the media at final concentration of 0.1, 0.2, 1, and 2 µM. The bacterial growth curve measurement was performed in a 48 well plates where we added the bacterial culture at a volume 0.3 ml/well with initial OD_{600} of 0.1. The plates were incubated at 37 ºC with agitation for 5 days. The growth was measured at certain important hours (1, 2, 3, 4, 5, 6, 7, 8, 24, 27, 30, 33, 48, 51, 54, 57, 72, 75, 78, 81, 96, 99, 102, 105, and 120 h) at OD_{600}.

Bacterial Biofilm Assay

Bacterial strains grown in TSB overnight at 37 ºC with agitation were used as preculture. The resuspended bacterial cells in sterile phosphate buffer were then re-inoculated into MKB at initial OD 0.1 medium supplemented with 10 µM or 50 µM of $FeCl_3$. The cultures were grown in 96 well plate with 200 µl culture of each well and incubated at 37 ºC without agitation for 72 h. After 72 h, the biofilm formation was measured according to previous publication (O'Toole, 2011). Briefly, after incubation, the supernatant was discarded carefully and the biofilm formed in the well was washed using phosphate buffer. Crystal violet solution (0.1%) was then added (200 µl) to stain the biofilm and incubate at room temperature for 1 h. The biofilm was then washed carefully to remove the unbound crystal violet. Methanol (200 µl) was added to the well to solubilize the bound crystal violet to the biofilm and the absorbance was measured at 550 nm.

Statistical analysis

The obtained growth curve data were analyzed statistically using Repeated Measures (RM) one-way

ANOVA followed by Tukey's multiple comparison test for the grouping. The data obtained from biofilm assays were analyzed using 2-way ANOVA and followed by Tukey's multiple comparison test.

Results and discussion

Dopamine at low concentration enhances the growth of S. aureus and S. epidermidis

In order to investigate the effects of dopamine on the iron availability and bacterial growth, we measured the siderophore activity of dopamine. The experiments showed that dopamine is able to chelate iron. At the concentration of 0.5 µM, dopamine can release Fe^{3+} from the CAS approximately 0.45 mM. However, although we added higher concentrations of dopamine, the released Fe^{3+} from CAS was never higher (**Fig. 1A**). We then analyzed the effects of dopamine on bacterial growth by supplementing dopamine into bacterial culture in iron-limited and iron-enriched media and observed the growth regularly for 5 days. In this study we used *S. aureus* USA300, *S. epidermidis* O47, and *E. coli* K12 as bacterial models representing human commensals. Growth curve experiments showed that dopamine supplementation significantly enhanced the growth of *S. aureus* USA300 only in iron-enriched media, but not under iron-limited conditions. (**Fig. 1B**). This tendency was not observed in either *S. epidermidis* O47 nor *E. coli* K12 (**Fig. 1C & 1D**).

Dopamine increases the biofilm formation

We also investigated the effect of dopamine supplementation on biofilm formation of these strains. In general, dopamine supplementation enhanced biofilm formation in all three bacterial strains under both iron-limited and iron-enriched conditions after 3 days of incubation. In *S. aureus* USA300, dopamine increased biofilm formation at a concentration of 1 µM in iron-limited media and 2 µM in iron-enriched media (**Fig. 2A**). *Staphylococcus epidermidis* O47 was more sensitive to dopamine, where in iron-limited media, 0.2 µM significantly increased biofilm formation, while in iron-enriched media, a significant increase was observed at 1 µM (**Fig. 2B**). *Escherichia coli* K12 showed similar tendency, where 1 µM of dopamine enhanced biofilm formation in both iron-limited and iron-enriched media (**Fig. 2C**).

Many studies reported the capability of dopamine in chelating iron (Porter et al., 2010; Dichtl et al., 2019). Given that dopamine concentrations in the human body range from 10 to 1000 nM (Liu & Liu, 2021), we investigated the extent of iron binding by dopamine at these physiological levels. Our results showed that, even at the physiological concentrations, dopamine showed significant iron

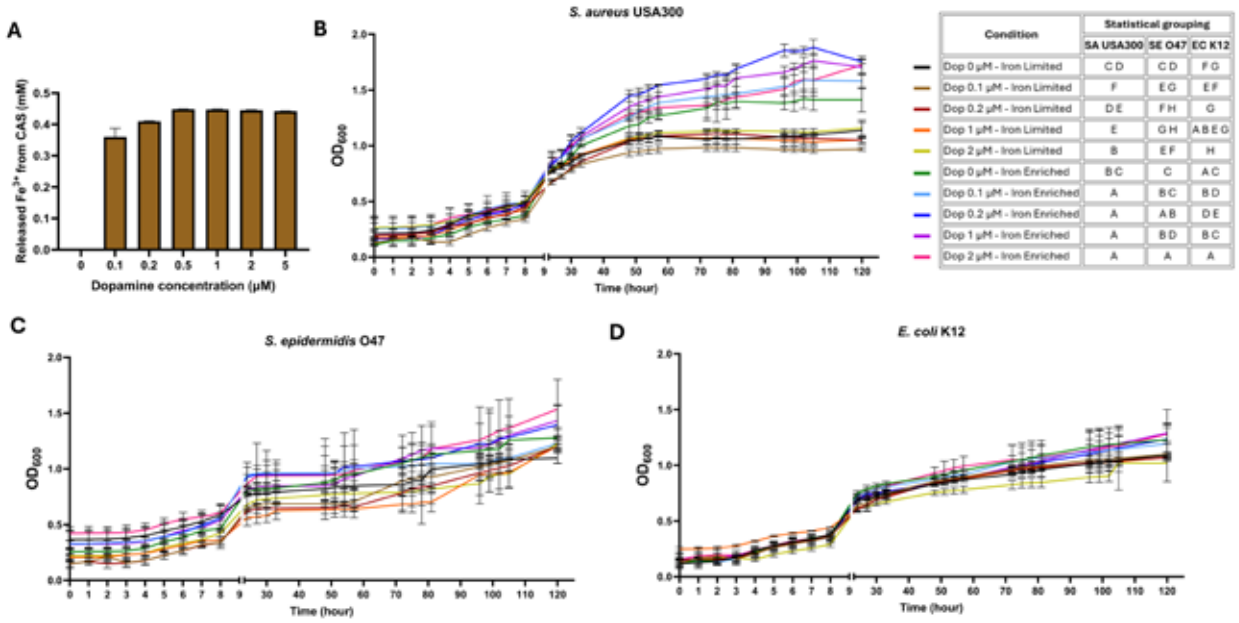

Fig. 1. Dopamine increases the growth of some bacteria due to its siderophore activity. (**A**) The CAS assays showed the capability of dopamine in chelating iron thus releasing iron from the CAS with the maximum released iron from the CAS was around 0.45 mM. The supplementation of dopamine enhances the growth of the iron enriched MKB medium in *S. aureus* USA300 (**B**) but no clear growth enhancement in *S. epidermidis* O47 (**C**) and *E. coli* K12 (D). For all graphs, each data point is the mean value ± SD (n=3). The statistical grouping was obtained by analyzing the growth data using Repeated Measures (RM) one-way ANOVA followed by the Tukey's multiple comparisons test. Dop: dopamine.

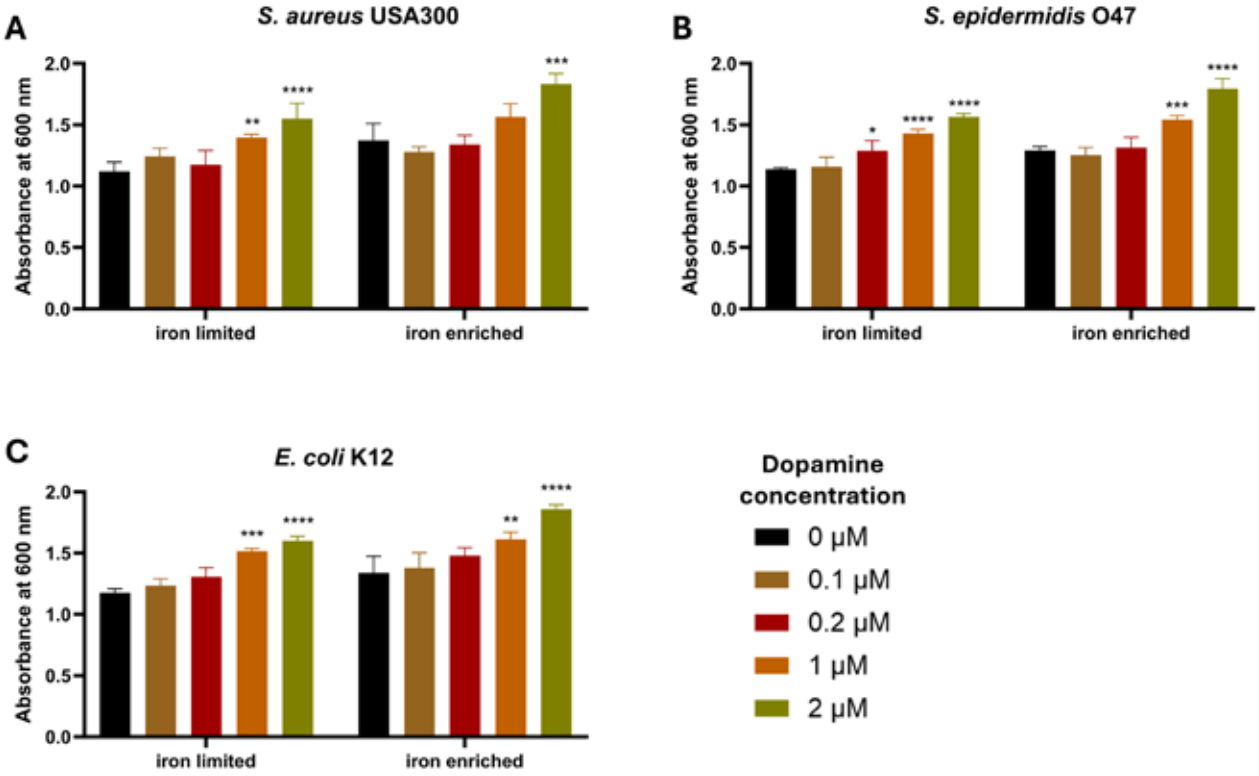

Fig. 2. Dopamine increases biofilm formation of bacteria. The biofilm formation assays of bacteria grown in iron-limited and - enriched MKB medium and incubated for 72 h, showed that dopamine increased biofilm formation in general at 1 µM for all three species: *S. aureus* USA300 (**A**), *S. epidermidis* O47 (**B**), and *E. coli* K12 (**C**). For all graphs, each data point is the mean value ± SD (n=3); **p*<0.05; ***p*<0.01; ****p*<0.001; and *****p*<0.0001, 2-way ANOVA followed by Tukey's multiple comparisons test were used to analyze the data.

chelating capability. However, the iron-binding capacity did not increase proportionally with higher dopamine concentrations, likely due to saturation of available iron, equilibrium between soluble and bound iron, kinetic limitations of iron binding to siderophores, and potential interference from dopamine autooxidation (Herlinger et al., 1995; Leventhal et al., 2016; Schiessl et al., 2017; Umek et al., 2018). Thus, the exposure of dopamine to human commensals or pathogenic bacteria in the case of infection could pose a significant effect.

The physiological concentration of dopamine increased the growth of *S. aureus* USA300 significantly in iron-enriched media but not in iron-limited media. This is most probably due to the capability of *S. aureus* to produce its own siderophore, such as staphyloferrin (Hammer & Skaar, 2011; Luqman & Ohlsen, 2024), which is sufficient to bind most of the available iron in the media. The presence of dopamine increased the iron availability for *S. aureus* in the iron-enriched media which in turn caused *S. aureus* to grow even more. Moreover, *S. aureus* has been reported to be able to utilize xenosiderophore for iron acquisition (Endicott et al., 2017). Different tendency was shown by *S. epidermidis*. No clear effect on *S. epidermidis* growth was observed with varying concentrations of $FeCl_3$ and dopamine in the media, likely due to its efficient ability to overcome severe iron limitation by utilizing multiple mechanisms for iron acquisition (Oliveira et al., 2017). A similar trend was observed in *E. coli*, which also demonstrates redundant iron acquisition systems (Garcia et al., 2011; Garenaux et al., 2011).

Biofilm formation is also greatly influenced by iron. The presence of dopamine in the media increased the biofilm formation at the concentration of 1 µM for all tested bacteria, which is still in the range of physiological dopamine level in human body (Liu & Liu, 2021). This implies that dopamine in human body might increase biofilm formation during bacterial infection. This could, in turn, increase the persistence of the infection (Soto et al., 2006; Sanchez et al., 2013) due to biofilm properties that help bacteria to resist antibiotic treatment and evade host immune responses (Otto, 2006; Hanke & Kielian, 2012; Le et al., 2018).

Conclusion

Here, we show that the physiological level of dopamine increases the growth of *S. aureus* USA300 in iron-enriched media but not in iron-limited media. Moreover, this physiological dopamine level enhances biofilm formation of *S. aureus* USA300, *S. epidermidis* O47, and *E. coli* K12. These findings provide an insight into the effect of dopamine on bacterial growth and biofilm formation in an *in vivo* condition as we used the physiological level of dopamine in human body.

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