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Original Research Article

L-Arginase from *P. aeruginosa* Isolated from Sewage as Anti-Biofilm Formation by Pathogenic Bacteria

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Abstract

Background: The enzyme arginase plays a significant role in the growth of many organisms as important biological enzyme. ***Objective*:** The study was designed to isolate L-arginase from P. aeruginosa that isolated from sewages and to evaluate the influence of arginase on biofilm-generating bacteria. ***Materials and Methods***: The current study includes 28(54.8%) isolates from soil with 24 (46.2%) from sewage water out of 52 isolated from soil and sewage for the purpose of obtaining *Pseudomonas aeruginosa*. Using standard laboratory methods to isolate *Pseudomonas aeruginosa*, 33 isolates were isolated, of which 15 (45.45%) were from soil and 18 (54.54%) from sewage. The study also included 177 different eyes (urinary fluid, excrement, blood, wounds, sputum, and cerebrospinal fluid) of patients suffering from various injuries. ***Results*:** The statistical analysis of the isolates indicated that there is a high significant (P≤0.01) for the growth of some types of bacteria, such as *P. aeruginosa 30*(16.89%), *Klebsiell*a, *Serratia* 33(18.88%) and *Acinetobacter 3*3(18.88%). While the results showed that *Lactobacillu*s is the lowest number of isolates 7(3.9%). Optimum condition of arginase activity and stability comprising pH level of eight degrees, the temperature of 37℃ is the best conditions for the production of arginine, concentrations of L-arginine substrate (0.1-2.5)g/l on the activity of arginase enzyme for arginine production, The finding also shows that the arginase enzyme exhibited the highest activity after 96 hours of the incubation process L-arginase Purification Assay including Ammonium Sulphate precipitation in (60-80)%, Dialysis, Ion exchange chromatography, Gel filtration**. *Conclusions*:** This study concluded that the enzyme arginase had a significant effect on the strong biofilm of pathogenic bacteria.

Keywords: *P. aeruginosa*, biofilm of pathogenic bacteria, purified L-arginase enzyme.

INTRODUCTION

L-arginase is an enzyme that catalyzes the hydrolysis of L-arginine to produce L-ornithine and urea. It is found in various organisms, including bacteria, fungi, and mammals, and productions a crucial part in the ruling of arginine metabolism. *P. aeruginosa* is a Gram-negative bacterium that is recognized for its versatile metabolic capabilities and its ability to cause opportunistic infections in humans. L-arginase has been identified and characterized *in P. aeruginosa*, as well as its part in arginine metabolism in this bacterium has been studied. One study by (Yu *et al*., 2009) [1] investigated the presence and regulation of L-arginase in *P. aeruginosa*. The researchers demonstrated that *P. aeruginosa* possesses a functional L-arginase enzyme and that its expression is influenced by environmental factors, such as the availability of arginine and the presence of other nitrogen sources, they also found that L-arginase activity in *P. aeruginosa* is important for bacterial growth under certain conditions.

Another study by (Zhang *et al.*, 2011) [2] focused on the role of L-arginase in *P. aeruginosa* virulence. The researchers found that L-arginase contributes to the production of polyamines, which are involved in various cellular processes and can enhance bacterial survival and pathogenicity. They showed that *P. aeruginosa* strains lacking L-arginase exhibited reduced virulence in a mouse model of acute lung infection.

Biofilm construction is a composite and coordinated development exhibited by many bacterial types. Biofilms stay structured societies of microorganisms enclosed in a self-produced

extracellular milieu, which allows them to adhere to various surfaces and protect themselves from environmental stresses. The formation of biofilms plays a critical part in the pathogenicity and persistence of bacteria in diverse settings, including medical devices, industrial systems, and natural environments [3].

Also review by (O'Toole and Wong 2016) [4] focuses on the sensory mechanisms employed by bacteria to initiate and regulate biofilm formation. The authors discuss the various signal transduction pathways involved in surface sensing and the coordination of gene expression during biofilm development. They also highlight recent advances in understanding the role of physical forces and mechanical cues in biofilm formation.

(Jawan *et al*., 2020) [5] focused on L-arginase production by *Lactobacillus* plantarum MTCC 1407 and investigated the influence of culture conditions on its production. The researchers optimized various parameters such as pH, temperature, inoculum size, and carbon and nitrogen bases using reaction surface methodology. They identified glucose and yeast extract as the ideal carbon and nitrogen bases for maximum L-arginase production [5].

Pathogenic bacteria play a significant role in causing various diseases in humans, animals, and plants. These disease-causing bacteria are capable of invading and colonizing the host's tissues, where they can cause damage and trigger immune responses. The danger posed by pathogenic bacteria is evident in their ability to spread rapidly, leading to outbreaks and epidemics if not effectively controlled. Here's an overview of their role and the associated risks [6, 7]:

- Toxin Production: Some pathogenic bacteria produce toxins that can directly damage host tissues and organs. For instance, Clostridium tetani produces tetanus toxin, which affects the nervous system, causing muscle stiffness and spasms. Similarly, Clostridium botulinum produces botulinum toxin, which leads to muscle paralysis. These toxins can have severe and potentially lethal effects on the host.
- Antibiotic Resistance: Pathogenic bacteria have the ability to acquire fighting to antibiotics, constructing infections further problematic to extravagance. Overemployment and abuse of antibiotics donate to the appearance and extent of antibiotic-resistant strains, posing a significant public health threat. Instances of antibiotic-resistant bacteria comprise methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant tuberculosis (MDR-TB).
- Epidemics and Outbreaks: Pathogenic bacteria have the potential to cause widespread outbreaks and epidemics. When conditions are favorable for their transmission, such as crowded environments or inadequate sanitation, these bacteria can rapidly spread from person to person. Outbreaks of bacterial infections, such as cholera, meningococcal meningitis, or foodborne illnesses, can result in a high number of cases and pose a important risk to community health.

The current study was designed to isolate L-arginase from P. aeruginosa that isolated from sewages and to assess the influence of arginase on biofilm-generating bacteria.

MATERIALS AND METHODS

Pseudomonas aeruginosa (soil and sewage) as well as the rest of pathological bacteria (urine, feces, blood, cerebrospinal fluid, wounds, and sputum) were isolated and diagnosed by the traditional methods used in laboratory diagnostics, which include culture media, gram stain, and biochemical tests with the use of the Vitek2PG system/England.

Primary detection *p. aeruginosa* production of L – arginase enzymes by streaking on M9 agar media form soil and swage. Primary detection p. aeruginosa production of L – arginase enzymes by streaking on M9 A agar media form soil and swage as explained by [8], and the optimum condition for L-arginases production was designated according to [9], that including effect of PH, temperature, substrate concentration, incubation Period and type of media [9].

L- arginase Purification Assay

Including Ammonium Sulphate precipitation, Dialysis, Ion exchange chromatography, Gel filtration Sephadex- S200.

Characterization of L-arginase purified

Thermal and pH (activity and stability)

RESULTS

52 bacterial isolates were obtained from different agricultural soils and sewage water, where the isolates consisted of 28 (53.8%) from soil and 24(46.2%) from sewage, as in Table (1)

Table 1: Distribution of samples from soil and sewage

Source	No.	Percentage %
Soil	28	53.8
Swage	24	48.2
Total	52	100%
Chi-Square (χ^2)	---	0.307 NS
P-value	---	0.579
NS: Non-Significant.		

From a entire of 52 bacterial isolates from soil and sewage, laboratory tests stayed conducted on them

in order to identify the species and types of these bacterial isolates, Where the outcomes displayed that the growth of *P. aeruginosa* 15(28.8%) and 18(34.6%) is highly significant (P≤0.01) more than the rest of the other species found in the soil and sewage water, respectively as in Table (2).

Table 2: Pseudomonas aeruginosa isolation from soil and sewage

Position	No.= 33	Percentage %
Soil	**15**	**45.45**
Sewage	**18**	**54.54**

Distributions of bacterial clinical according to isolated source

177 different samples were collected from patients inside the hospital. The samples included faeces, urine, wounds, blood and sputum, in addition to cerebrospinal fluid where they were classified according to the bacterial growth resulting from each isolate. Statistical analysis of the isolates indicated that there is a high significant (P≤0.01) for the growth of some types of bacteria, such as P. *aeruginosa*30 (16.89%), *Klebsiella*, *Serit*a, 33 (18.88%) *and Acinetobacter* 33 (18.88%), while the results showed that *Lactobacillus* is the lowest number of isolates 7(3.9%), Table (3).

Table 3: Distributions of bacterial clinical according to isolated source

Name of Bactria	Urine No.%	Stool No.%	Sputum No. %	Wound No.%	Blood No.%	Csf No. %	Total	Total Percentage %	P-value
pseudomonas aeruginosa	4/13.33%	-	6/20%	16/ 53.31	4/13.33	-	30	16.89%	0.0084 **
Serrati Marcescens	18/54.54	-	-	8/24.43%	7/21.2%	-	33	18.88%	0.0002 **
Klebsiella pneumonia	10 30.1	2 6.1	7 21.3	8 24.3	6 18.2	-	33	18.88 %	0.034 *
Acinetobacter baumannii	-	-	8/26.6%	13/26.6%	8/26.6%	1/3.3	30	16.8%	0.0094 **
Escherichia coli	5/41.66%	2/16.66%	1/8.33%	3/25%	1/8.33%	-	12	6.7%	0.094 NS
staphylococcus aureus	4/33.23%	-	1/8.33%	-	2/16.66%	5/41.66	12	6.7%	0.105 NS
streptococcus pneumonia	3/30%	-	-	5/50%	2/20%	-	10	5.7 %	0.227 NS
Lactobacillus ssp	7 100%	-	-	-	-	-	7	3.9%	0.049 *
Morganella morganii	3/30%	-	5/50%	2/20%	-	-	10	5.64%	0.308 NS
Total	54	4	28	55	30	6	177	100%	---
**** (P≤0.05), ** (P≤0.01).***									

Optimum condition of arginase activity and stability

Table 4: Optimum condition of arginase activity and stability

Condition	Effective range
pH	8
Temperature	37℃
Concentration of substrate	(0.1-2.5) g/l
Time of incubation	96 hours
Media	beef extraction yeast extraction medium

Purification of L-arginase enzyme fromaer*uginosa* isolation sample

Table 5: Purification of L-arginase enzyme from *aeruginosa* isolation sample

Purification step	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification fold	Yield %
Crude enzyme	250	4.058	11.144	0.364	1014.5	1	100
Precipitation with 80% saturation of (NH4) SO_4	30	5.019	10.056	0.499	157.57	1.371	14.841
desalting and concentration by dialysis	20	4.750	9.213	0.516	95	1.418	9.364
Ion exchange chromatography (DEAE-Cellulose)	25	3.343	3.416	0.979	83.575	2.690	8.238
Gel filtration G200	25	2.861	3.271	0.875	71.525	2.404	7.05

Biofilm activity (week, moderate and strong) of pathogenic bacteria

Table 6: Biofilm activity (weak, moderate and strong) of pathogenic bacteria

Strongly adherent %	Moderately adherent %	Weakly adherent %	Non. Adherent %	No. of isolates	Bactria
2 6.6	13 43.3	5 16.6	10 33.3	30	*p.aeruginosa*
6 18.20	8 24.24	10 30.3	9 27.27	33	*s. marcescens*
3 9.09	14 42.44	6 18.20	10 30.3	33	*k. pnemonai*
2 6.7	17 56.6	0 -	11 36.7	30	A. b*aumannii*
0	6 50	2 16.7	4 33.3	12	s. aureus
1 10	6 60	0 -	3 30	10	S. *pnemonia*
1 8.3	- -	7 58.4	4 33.3	12	*E.coli*
15 9.4	64 40	30 18.7	51 31.9	160	Total
0.048 *	0.0074**	0.0394 *	0.227 NS		P-value
**** (P≤0.05). ** (P≤0.01)***					

Detection of bacteria that produce biofilm by using Congo red method

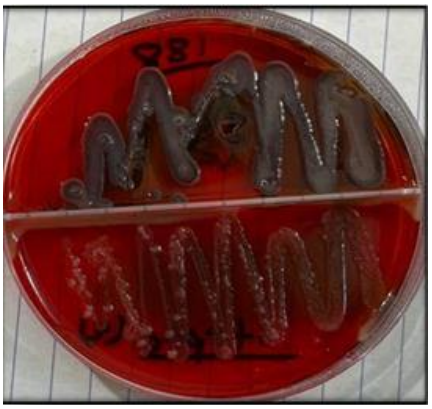

Figure 1: Detection of *Klebsiella spp.* by using congo red method

Primary detection *p. aeruginosa* production of L – arginase enzymes by streaking on M9 agar media form soil and swage

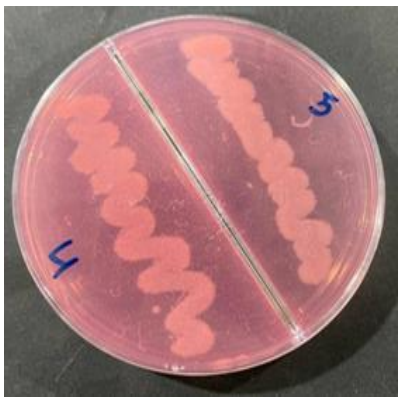

Figure 2: Primary detection *p. aeruginosa* production of L – arginase enzymes by streaking on M9 agar media form soil and swage

MIC (minimum inhibiter concentration) from L-arginase enzyme against pathogens bacterial

The present results showed that the effect of the minimum inhibitory concentration of arginase enzyme was more effective and significant for pathogenic bacteria isolated from wastewater (1.139U/ml) than pathogenic bacteria isolated from soil (1.541U/ml).

Enzyme activity on pathogenic bacteria strong biofilm (as anti-biofilm)

The current results showed that the effectiveness of arginase enzyme is highly significant in reducing biofilm formation against biofilm-producing bacteria in pathogenic isolates that were treated with this enzyme more than in bacteria that were not treated with this type of enzyme.

Table 7: Enzyme activity on bacterial strong biofilm

Name of Bactria	Boiflimwith out enzyme(630)	SD	Boiflim with enzyme 18s(630)	SD	Boiflim with enzyme 20m(630)	SD
And	0.985	0.26	0.364	0.08	0.515	0.08
K	0.239	0.07	0.038	0.01	0.195	0.04
Sm	1.256	0.34	1.134	0.13	0.753	0.08
AND	0.724	0.19	0.229	0.04	0.050	0.02
Ec	0.292	0.08	0.005	0.00	0.036	0.01
st	0.440	0.08	0.228	0.04	0.370	0.02
srt	0.233	0.05	0.070	0.02	0.038	0.01
Control	0.123	0.06	0.250	0.07	0.130	0.03
P-value	0.0001 **	---	0.0001 **	---	0. 0092 **	---
***** (P≤0.01).***						

DISCUSSION

The current results showed that there were no significant differences in the number between the isolates from soil and sewage, although the number of isolates in the soil is more than the isolates in sewage water, as the presence of bacteria in the soil is equivalent to more than any microorganism, given that it has the ability to multiply in small particles. Soil and near the roots of plants and quickly despite the change of environmental conditions in the soil and this is consistent with [10]. In addition, sewage water constitutes a good environment for the growth of many pathological and non-pathological bacteria, because it contains many elements necessary for the growth and reproduction of these bacterial species, and this is consistent with [11].

Statistical analysis of the isolates indicated that there is a high significant (P≤0.01) for the growth of some types of bacteria, such as *Pseudomonas aeruginosa30* (16.89%), *Klebsiella*, *Seritia*, 33(18.88%) and *Acinetobacter* 33(18.88%). While the results showed that *Lactobacillus* is the lowest number of isolates 7(3.9%), this agree with [12], who almost concluded that these species are the most common in the clinical pathogenic isolates of the various samples included in the current study.

The pH of a solution plays a crucial role in enzyme activity and stability. Enzymes have an optimal pH range at which their catalytic activity is highest. Deviation from this optimal pH can result in reduced enzyme activity and, consequently, decreased product formation. In the case of arginine production, the observed peak at an acidity level of eight degrees suggests that the enzymatic reactions involved in arginine synthesis are most favorable at this pH agree with [13].

This study is consistent with [14] where it was found that this degree is ideal for the construction of many amino acids, including arginase, where the temperature is one of the greatest significant features affecting the growing of microorganisms. The concentration of substrate can significantly influence enzyme activity and stability the subsequent production of desired metabolites. Enzymes often exhibit substrate specificity and may require an optimal substrate concentration for efficient catalysis. In the case of arginase, which is responsible for the alteration of L-arginine, the concentration of the substrate L-arginine can affect the enzymatic activity. Previous studies have explored the impact of substrate concentration on arginase activity and arginine production. For example, research on arginase activity in microorganisms has shown that the enzyme's action is influenced by the concentration of L-arginine substrate [15].

One possible explanation for the increased activity of arginase after 96 hours is the accumulation of enzyme protein due to continuous synthesis. Previous studies have shown that enzymes can be synthesized and accumulate over time in response to specific stimuli or environmental conditions [16].

Purification of L-arginase enzyme from *p.aeruginosa*

Sewage is a complex environment that contains diverse microorganisms, including bacteria from human and animal waste, industrial effluents, and other sources. These microorganisms may possess different metabolic capabilities and adaptations to the sewage environment, which can impact the expression and activity of arginase.

Additionally, the presence of various nitrogen-containing compounds in sewage, such as proteins and amino acids, may provide abundant substrates for arginase activity. On the other hand, soil is a heterogeneous environment with its own unique microbial communities. The microbial population in soil is predisposed via features for instance organic matter content, pH, moisture, and nutrient availability [17].

The precipitation in ammonium sulfite phenomenon can occur due to various factors such as changes in temperature, pH, or the presence of specific salts or ions in the solution [18]. The usage of ammonium sulfite precipitation followed by sucrose dialysis is a common method for enzyme concentration and purification. The higher arginase activity observed after concentrating the precipitate suggests successful recovery of the enzyme from both the soil and sewage isolates [19].

Ion exchange is a widely used method for enzyme purification and separation based on the differential affinity of enzymes for specific ion exchange resins. The observed difference in arginase activity between wastewater and soil isolates suggests that the enzyme may have a higher affinity or binding capacity for the ion exchange resin in the wastewater isolates, leading to a greater recovery of active enzyme [20, 21].

Gel filtration chromatography, likewise recognized as size elimination chromatography, separates particles founded on their size and molecular weight. The elution volume represents the point at which the protein of interest is eluted from the gel filtration column. The decrease in arginase enzyme activity with increasing elution volume could be attributed to several factors. One possibility is that the enzyme undergoes conformational changes or interactions with other molecules during the chromatographic process. These changes in the enzyme structure or interactions may affect its catalytic activity and reduce the observed enzymatic activity in the eluted fractions [22].

Another research article titled "Role of Arginase in Inhibition of Biofilm Formation by *Streptococcus mutans*" by [23] supports the effectiveness of arginase against biofilm-producing bacteria. In this study, the researchers focused on *Streptococcus mutans*, a key bacterium associated with dental plaque and tooth decay.

CONCLUSION

The current study concluded that Pseudomonas aeruginosa is one of the important bacterial species from which we can extract the arginase enzyme, which needs appropriate and accurate conditions to increase its production in these bacterial species, since these conditions production significant part in the production of the enzyme. The arginase enzyme is an important factor against the strong and effective biofilm in many pathogenic bacterial species that produce this biofilm.

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LIST OF ABBREVIATIONS

Abbreviations	Expansion
C°	Celsius
DW	Distiled water
pH	Power of Hydrogen
MDR – TB	multidrug-resistant tuberculosis
MRSA	methicillin-resistant St*aphylococcus* aureus
MIC	(minimum inhibiter concentration

REFERENCES

1. Yu, H., Schurr, M. J., & Deretic, V. (1995). Functional equivalence of Escherichia coli sigma E and Pseudomonas aeruginosa AlgU: E. coli rpoE restores mucoidy and reduces sensitivity to reactive oxygen intermediates in algU mutants of P. aeruginosa. *Journal of bacteriology*, *177*(11), 3259-3268.
2. Zhang, L., Jia, X., & Zhang, L. (2011). The contribution of a Pseudomonas aeruginosa arginine deiminase in polyamine biosynthesis to oxidative stress resistance and virulence. *Molecules*, *16*(6), 4499-4515.
3. Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *science*, *284*(5418), 1318-1322.
4. O'Toole, G. A., & Wong, G. C. (2016). Sensational biofilms: surface sensing in bacteria. *Current opinion in microbiology*, *30*, 139-146.
5. Jawan, R., Abbasiliasi, S., Tan, J. S., Mustafa, S., Halim, M., & Ariff, A. B. (2020). Influence of culture conditions and medium compositions on the production of bacteriocin-like inhibitory substances by Lactococcus lactis Gh1. *Microorganisms*, *8*(10), 1454.
6. Balloux, F., & van Dorp, L. (2017). Q&A: What are pathogens, and what have they done to and for us?. *BMC biology*, *15*(1), 1-6.
7. Vestby, L. K., Grønseth, T., Simm, R., & Nesse, L. L. (2020). Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics*, *9*(2), 59.
8. LaBauve, A. E., & Wargo, M. J. (2012). Growth and laboratory maintenance of Pseudomonas aeruginosa. *Current protocols in microbiology*, *25*(1), 6E-1.

9. Keni, S., & Punekar, N. S. (2016). Contribution of arginase to manganese metabolism of Aspergillus niger. *Biometals*, *29*, 95-106.
10. Haas, D., Lesch, S., Buzina, W., Galler, H., Gutschi, A. M., Habib, J., ... & Reinthaler, F. F. (2016). Culturable fungi in potting soils and compost. *Sabouraudia*, *54*(8), 825-834.
11. Aziz, A. M., & Aws, A. (2012). Waste Water Production Treatment and use in Iraq Country report. Republic of Iraq Ministry of Water Resources. 1-21. https://www.ais.unwater.org/ais/pluginfile.php/356/mod_page/content/114/Iraq_WasteWaterProduction.pdf
12. Siqueira Jr, J. F., & Rôças, I. N. (2013). Microbiology and treatment of acute apical abscesses. *Clinical microbiology reviews*, *26*(2), 255-273.
13. Lowe TL, Clarke SG. Human protein arginine methyltransferases (PRMTs) can be optimally active under nonphysiological conditions. J Biol Chem. 2022; 298(9): 102290. (PMID: 35868559).
14. Matysiak, K., Kierzek, R., Siatkowski, I., Kowalska, J., Krawczyk, R., & Miziniak, W. (2020). Effect of exogenous application of amino acids l-arginine and glycine on maize under temperature stress. *Agronomy*, *10*(6),769. https://www.mdpi.com/2073-4395/10/6/769
15. Deshpande, N., Choubey, P., & Agashe, M. (2014). Studies on optimization of growth parameters for L-asparaginase production by Streptomyces ginsengisoli. *The Scientific World Journal*, *2014*.
16. Dunphy, K., Dowling, P., Bazou, D., & O'Gorman, P. (2021). Current methods of post-translational modification analysis and their applications in blood cancers. *Cancers*, *13*(8), 1930.
17. Wongkiew, S., Chaikaew, P., Takrattanasaran, N., & Khamkajorn, T. (2022). Evaluation of nutrient characteristics and bacterial community in agricultural soil groups for sustainable land management. *Scientific reports*, *12*(1), 7368.
18. Kim, K. Y., Lee, J. K., & Ahn, B. Y. (2019). Metagenomic analysis of microbial communities in a packed-bed anaerobic reactor for the treatment of malodor and volatile organic compounds. *J Microbiol Biotechnol*, *29*(2), 298-307. https://www.sciencedirect.com/science/article/abs/pii/S0960852421019544
19. Wingfield, P. (1998). Protein precipitation using ammonium sulfate. *Current protocols in protein science*, *13*(1), A-3F.
20. Liu, Y. C., ChangChien, C. C., & Suen, S. Y. (2003). Purification of penicillin G acylase using immobilized metal affinity membranes. *Journal of Chromatography B*, *794*(1), 67-76.
21. Zhang, Y., Cui, D., Yang, H., & Kasim, N. (2020). Differences of soil enzyme activities and its influencing factors under different flooding conditions in Ili Valley, Xinjiang. *PeerJ*, *8*, e8531.
22. Ó'Fágáin, C., Cummins, P. M., & O'Connor, B. F. (2017). Gel-filtration chromatography. *Protein Chromatography: Methods and Protocols*, 15-25.
23. Chakraborty, B., & Burne, R. A. (2017). Effects of arginine on Streptococcus mutans growth, virulence gene expression, and stress tolerance. *Applied and environmental microbiology*, *83*(15), e00496-17.