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ORIGINAL ARTICLE

Effects of genetic and environmental variables on biofilm development dynamics in *Achromobacter mucicolens*

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ABSTRACT

Background and Objectives: The study aimed to investigate whether *Achromobacter mucicolens* IA strain biofilm formation, which contributes to antibiotic resistance, could be enhanced by readily available nutrient sources like carbohydrates and environmental factors such as pH and NaCl. Additionally, the study aimed to identify any inherent genes that support biofilm formation in this strain, which is an opportunistic pathogen that affects immunocompromised patients and is resistant to many antibiotics.
Materials and Methods: Biofilm growth in different carbohydrate, pH, and NaCl concentrated media was measured using crystal violet microtiter assay. All the treatments were subjected to biostatistics analysis for normality, Test of Homogeneity, one way ANOVA analysis. Whole-genome sequencing of our IA strain was conducted to identify various gene sequences.
Results: Biofilm formation was measured at different carbohydrate concentrations, and the optimum biofilm formation was observed at 3M glucose and 0.5M NaCl, while the lowest results were seen at 2M maltose concentration. Whole-genome sequencing identified potential genes involved in biofilm formation, pathogenicity, protein metabolism, flagellar motility, cell wall component synthesis, and a multidrug efflux pump.
Conclusion: These findings suggest that biofilm formation is influenced by extrinsic and intrinsic factors, which could aid in the development of effective treatments for resistant infections.

Keywords: *Achromobacter mucicolens*; Biofilms; Carbohydrates; Genome, Bacterial; Drug resistance; Bacterial; Pathogenicity

INTRODUCTION

Members of the genus *Achromobacter* are lactose non-fermenting, Gram-negative bacteria rods that may grow in both aerobic and anaerobic settings, despite their aerobic classification, in natural aquatic sources (1). Apart from their natural habitats, they easily grow in hospitals and in immunity-weak individuals. In immunocompromised persons, invasive *Achromobacter* infections can be lethal. *Achromobacter* species also have the potential to induce both nosocomial and community-acquired illnesses. Most

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infections occur during hospitalization, with primary simple bacteremia, catheter-associated infections, and pneumonia being the most prevalent presentations (2). Certain species of *Achromobacter*, most notably *xylosoxidans* and *denitrificans*, can, however, cause disease in specific patient populations, including those with cystic fibrosis, hematologic and solid organ cancers, renal failure, and immunodeficiencies (3-5).

Recently, it has been acknowledged that the capacity of *Achromobacter* species to generate biofilms is a crucial factor in their ability to cause disease, and it is predicted that this factor will significantly contribute to the maintenance of infections caused by specific strains of *Achromobacter* in susceptible populations (6-8). The creation and growth of bacterial biofilms are known to be dynamic and complicated processes regulated by intrinsic biological features as well as various extrinsic influences, as changes in the environment frequently drive biofilm formation (9). The tendency of the *Achromobacter* subspecies to bind to abiotic surfaces and host tissues and form biofilms has been explored as a characteristic trait (10). The genes involved in biofilm development, antibiotic resistance, and motility are all regarded as important prerequisites for *in vivo* colonization and infection, and recent research suggests that they are all interdependent (11-14).

Effective therapy can be challenging due to the *Achromobacter* subspecies *mucicolens*' intrinsic and acquired multidrug resistance characteristics. Furthermore, there are currently no consensus standards for *in vitro* biofilm quantification for *A. mucicolens* bacterial isolates based on biofilm-forming potential. Given the possibility that the strain under the study *A. mucicolens* – IA strain, produces biofilm to develop enhanced resistance to the host immune system, antibiotics, and other treatments, identifying isolates with such abilities would help researchers get a deeper perspective of the organism's pathophysiology. The purpose of this study was to see how biofilm-related genes, as well as environmental elements, affect the strain's ability to produce biofilms, in a clinical *A. mucicolens* IA strain isolate.

MATERIALS AND METHODS

Collection of samples. *Achromobacter mucicolens* was isolated from the sputum sample of a 47-year-old leukemia patient at Baghdad teaching hospital mixed with fungal growth in a patient with persistent cough with colored sputum, non-responding for multiple antibiotic drug combination therapy in the haemato-oncology Department. This sample was collected and *A. mucicolens* bacteria were cultured on McConkey agar plates further obtained pure cultures were used for its identification, drug resistance tests, and ultimately full genome was sequenced.

***In vitro* biofilm production.** The sputum sample was collected from a respiratory infection patient and then cultured overnight on nutrient and MacConkey agar at 37°C. After that, a pure colony of *A. mucicolens* from an overnight grown agar culture plate was inoculated into 10 ml of LB (Luria Bertini) broth and incubated at 37°C overnight. By preparing technical replicates, the culture was adjusted to 0.64 McFarland (DensiCHEL Plus Instrument, Biomerieux) used to estimate the concentration of bacterial cells in a liquid culture, and 200 µl of adjusted cell suspension was placed in each well of the 96 plates and incubated at 37°C for 24 hours in a moist chamber. Following incubation, the culture was carefully withdrawn from each well using a multichannel pipette. Before fixing, the wells were cleaned twice with 250 µl of distilled water to eliminate planktonic bacteria. For staining, 250 µl of 0.2M crystal violet was applied to each well at room temperature for 10 minutes. Finally, each well was gently rinsed 2-3 times with distilled water and allowed to dry at room temperature to eliminate excess crystal violet. The biofilm was resuspended in 200µl of 95% ethanol for elution, and optical density (OD) was measured in an ELISA plate reader at a max of 595nm (Thermo Scientific, USA) (15).

Using a microtiter plate, the results of biofilm formation were predicted based on the OD value of sterile LB broth with crystal violet and sugar from each experiment as a negative control.

Carbohydrate and salt supplementation. Glucose, sucrose, maltose, galactose, starch, lactose, and fructose were tested at concentrations ranging from 1M to 4M, with NaCl concentrations ranging from 0.5 to 3.5M and pH ranging from 4 to 8, respectively to examine the effect of maximal biofilm yielded by supplementation.

Genome sequencing, assembly, and annotation. The Presto Mini gDNA Bacterial Kit (Geneaid) was

used to extract genomic DNA from loopful bacterial cells according to the manufacturer's instructions. The bacterial taxonomy was identified using the biochemical test VITEK-2 (bioMérieux Vitek Systems Inc., Hazelwood, MO) which identified the bacteria as *Achromobacter mucicolens.* The complete genome sequence was done by using sequencing by synthesis (SBS) technology, Next-generation sequencing (NGS) technology on the Illumina platform (Illumina Inc.) at Macrogen, Korea. The whole-genome assembly was evaluated using FastQC v0.11.7 and was later annotated using the software RAST 2.0 to obtain gene details, coding regions, and protein information (16-18). Molecular analysis by next-generation sequencing was carried out to identify the novel whole genome sequence of the *A. mucicolens* strain, and detect various drug resistance genes and other unique genomic features of this bacterial strain studied with advanced bioinformatic tools as previously described in our research by Sura Ali Al-Asadi et al. (19).

Data availability. The entire genome of the *A. muciolens* strain was sequenced using Illumina's Next-generation sequencing platform (Novoseq6000) and submitted to GenBank under the BioProject number PRJNA224116. The Accession number for this study is CP082965, and the version mentioned in this paper is CP082965. This *A. mucicolens* strain is known as the IA strain based on its subspecies categorization.

Statistical analysis. The differences in OD values obtained with different experimental variables of the *in vitro* synthesis of biofilm by Tissue Culture Plate (TCP) technique were computed and analyzed using a one-way analysis of variance and the conditions of the analysis of variance were verified as follows:

1-The condition that the data are subject to a normal distribution Tests of Normality: This condition was verified using Kolmogorov-Smirnov, and it became clear that the data of all the parameters were subject to the normal distribution, where the P-value was greater than 0.05 for all parameters.

2- Test of Homogeneity: This condition was verified using Levene Statistic and it turned out that all the variables are Levene Statistic where the P-value is greater than 0.05 for all parameters

3- The results of the one-way analysis of variance to test the significance of differences in the averages of Biofilm between the concentrations of different treatments

4-The analysis was carried out using the SPSS software and graph pad prism v9 software, IBM (20).

Bioinformatics analysis. The whole genome of the *A. mucicolens* IA strain was subjected to RAST 2.0 (16-18) for gene annotation to detect the existence of possible genes implicated at various phases that may play diverse roles in biofilm development, stability, and virulence. These various genes were investigated for their potential roles in biofilm development on other *Achromobacter* strains of *A. insolitus* and *A. xylosoxidans* (Table 1). These genes linked to biofilm development have been curated from Li et al., Nielsen SM et al. (21-23).

Ethics approval. We declare that the Ethical Committee of the institution in which the work was done has approved the study, and that the subjects gave informed consent to the work.

RESULTS

The following results were obtained for various factors on *in vitro* biofilm synthesis using the TCP test in order to achieve the conditions required for maximum biofilm biomass generated by *A. mucicoelns.*

Effect of glucose. When LB broth was treated with glucose, the clinical isolate developed a biofilm-positive phenotype. glucose was found to favorably contribute to biofilm development at practically all concentrations, however, the maximum absorbance was seen at 3M glucose as shown in Fig. 1a. The results revealed that there were differences between glucose concentrations in the biofilm mean, where the F value is 55.065, which is statistically significant at the level of significance of 0.01. To determine the best concentration, Duncan's test was used. It was found that the best glucose concentration is 3%, then 2%, 1%, then 4%.

Effect of lactose. It was noted the clinical isolate displayed a biofilm-positive phenotype when LB broth was supplemented with lactose. Its maximal absorbance was found at a concentration of 4M as shown in Fig. 1b. The results showed that there were differences between lactose concentrations in the biofilm mean, where the F value was 19.505, which is statistically significant at the level of significance of 0.01. To

Table 1. Main pathogenic determinants identified in *A. mucicolens.*

Product/Function	Gene Name/abbreviation
Protein Secretion	
TypeI	
Outer membrane component	LapE(BUW96_01540)
Membrane fusion protein	LapC(BUW96_15170)
Type I secretion system ATPase, LssB family	LapB(BUW96_15220)
T1SS-associated transglutaminase-like cysteine proteinase	LapP(BUW96_15220)
T1SS secreted agglutinin RTX	-(BUW96_01515)
Type II	
Flp pilus assembly protein	RcpC/CpaB(BUW96_11460, BUW96_11480)
Type II/IV secretion system protein, associated with Flp pilus assembly	TadC(BUW96_01430, BUW96_11455)
Type II/IV secretion system secretin, associated with Flp pilus assembly	RcpA/CpaC(BUW96_01415, BUW96_11475)
Type II/IV secretion system ATP hydrolase, TadA subfamily	TadA/VirB11/CpaF(BUW96_01420, BUW96_11465)
General secretion pathway	[a]Type C,D,E,F,G,H,I,J,K,L,M,N
Type VI	
ClpB protein	ClpB(BUW96_10310, BUW96_25090, BUW96_29740)
Sigma-54-dependent transcriptional regulator	-(BUW96_11435)
Uncharacterized protein ImpA	ImpA(BUW96_25135, BUW96_29605)
Uncharacterized protein ImpB	ImpB(BUW96_25130, BUW96_29710)
Uncharacterized protein ImpC	ImpC(BUW96_25125, BUW96_29715)
Protein ImpG/VasA	ImpG(BUW96_25105, BUW96_29730)
Uncharacterized protein ImpH/VasB	ImpH(BUW96_25095, BUW96_29735)
Uncharacterized protein ImpI/VasC	ImpI(BUW96_25085)
Denitrification	
Respiratory nitrate reductase alpha chain (EC 1.7.99.4)	-(BUW96_04895)
Respiratory nitrate reductase gamma chain (EC 1.7.99.4)	-(BUW96_04910)
Respiratory nitrate reductase delta chain (EC 1.7.99.4)	-(BUW96_04905)
Respiratory nitrate reductase beta chain (EC 1.7.99.4)	-(BUW96_04900)
Nitrate/nitrite transporter NarK	NarK(BUW96_04885, BUW96_04890)
Flagellar motility	
Flagellar motor proteins	FliF, FliM, FliN
Cell wall and capsule	
Capsular polysaccharide ABC transporter	KpsT, KpsM, KpsE
Virulence	
Arsenic resistance	ArsH
Cobalt-zinc-cadmium resistance	Nucleotide sequence present
Antimicrobial susceptibility	
Beta-lactamase	$Bla_{\text{OXA-10}}$
Multidrug resistance efflux pumps	AxyEF-OprN
Resistance-nodulation-cell division (RND)-type multidrug resistance efflux	MacA and MacB
Multidrug resistance	MexB, MdtA,B,C,G,H,K,L
Stress response	
Cold shock	CspA/CspG
Osmotic stress	OsmY
Potassium metabolism	
Osmosensitive K+ channel histidine kinase	KdpD II, III, IV, V
DNA metabolism	
DNA recombination	RuvA/RuvC

Table 1. Continuing...

DNA repair	RecO
Protein metabolism	
Protein biosynthesis	PurH, ArgJ, MetXA
Protein processing and modification	MsrA and MrsB
Biofilm Formation genes	
Chemotaxis regulator	BdIA, WspC

determine the best concentration, Duncan's test was used. There is higher morale between them.

Effect of galactose and fructose. When LB broth was added with galactose and fructose, respectively, the clinical isolate revealed a biofilm-positive phenotype. At all concentrations, galactose and fructose formed considerable biofilms, however, the greatest absorbance was seen at a concentration of 1M as shown in Figs. 1c and 1d. It was also observed that the absorbance decreased with increasing concentrations when treated with galactose. The results showed that there were significant differences between the concentrations of fructose and galactose in the Biofilm averages, where the F value is 27.770 and 137.523, which is statistically significant at a significant level of 0.01. To determine the best concentration, Duncan's test was used. It was found that the best concentration of fructose and galactose was 1%, then 2%, 3%, and 4%, and there was no significant difference between them.

Effect of starch. The clinical isolate showed a distinct biofilm-positive phenotype when LB broth was treated with starch. At virtually all concentrations, starch was found to favorably contribute to biofilm growth. The highest absorbance was seen at 1M starch as shown in Fig. 1e. The results showed that there are differences between the concentrations of starch in the biofilm averages, where the value of F reached 109.788, which is statistically significant at the level of significance of 0.01 and to determine the best concentration, the Duncan test was used and it was found that the best concentration of starch is 1%, 2%, then 4%, then 3%.

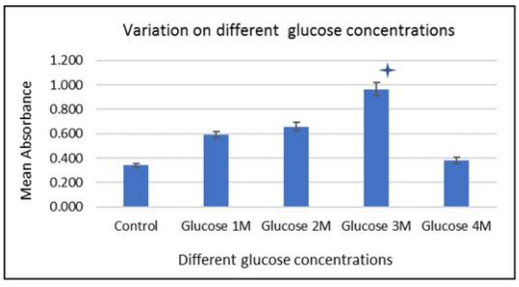
Effect of sucrose and maltose. The clinical isolate exhibited a biofilm-positive phenotype when LB broth was supplemented with sucrose and maltose, respectively. The maximum absorbance was seen at a concentration of 2M as shown in Fig. 1f and 1g). The results revealed that there were differences between maltose and sucrose concentrations in the biofilm mean, where the F value is 22.634 and 27.179, which is statistically significant at the level of significance of 0.01. To determine the best concentration, Duncan's test was used. It was found that the best maltose and sucrose concentration is 2%, then 1%, 4%, and then 3%. And there was no higher morale among them.

Effect of sodium chloride and pH. The supplementation of NaCl to the *A. mucicolens* isolate enhanced the biofilm phenotype, with the greatest absorbance seen at 0.5% NaCl as shown in Fig. 2. The results showed that there were differences between the concentrations of NaCl in the biofilm averages, where the value of F was 20.170, which is statistically significant at the level of significance of 0.01. To determine the best concentration, Duncan's test was used. It was found that the best concentration of NaCl is 0.5, 1.5, 1,3, 2, 2.5, and 3.5.

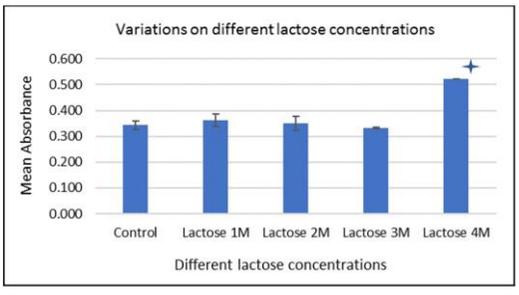
The optimal pH values for *A. mucicolens* biofilm growth are between 4 and 5.5, and pH values were statistically significant when LB broth was supplemented to maintain varied pH levels as shown in Fig. 3. The results showed that there were differences between the concentrations of pH in the biofilm averages, where the value of F was 23.189, which is statistically significant at the level of significance of 0.01. To determine the best concentration, Duncan's test was used. It was found that the best concentration of pH is 4, 5.5, 5, 8, 6, 7.5, 6.6.

Effect of the best treatment. The supplementation with different carbohydrate elements to the *A. mucicolens* had no significant effect on absorbance between carbon substrate at a temperature of 37ºC. The best treatment was seen at a concentration of 3M glucose with an average of 0.964, then NaCl 0.5M with an average of 0.907, and the lowest results were at 2M Maltose concentration as shown in Fig. 4.

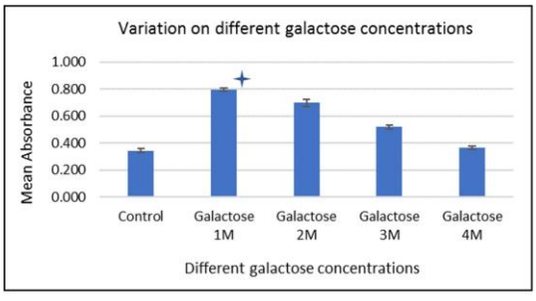
General genome characteristics. The genome is 5.89 bp in length and has a GC content of 65.9%. After



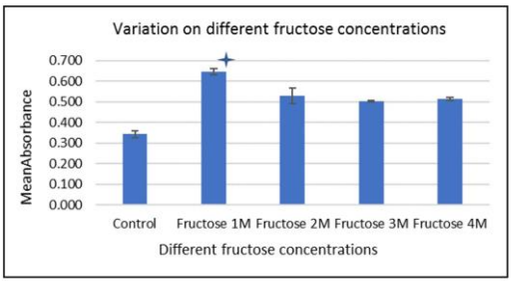
a. Effect of different concentrations of glucose supplementations on absorbance



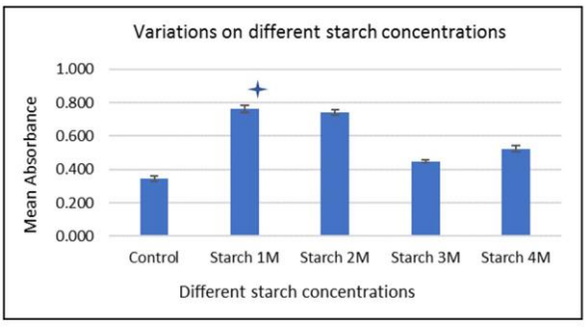
b. Effect of different concentrations of lactose supplementations on absorbance



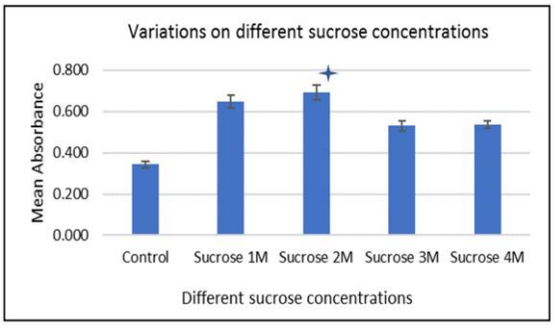
c. Effect of different concentrations of galactose supplementations on absorbance



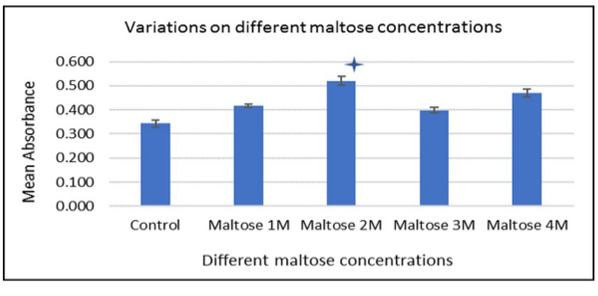
d. Effect of different concentrations of fructose supplementations on absorbance



e. Effect of different concentrations of starch supplementations on absorbance



f. Effect of different concentrations of sucrose supplementations on absorbance.



g. Effect of different concentrations of maltose supplementations on absorbance

Fig. 1. Effect of different concentrations of sugar supplementations on absorbance
Significant difference marked ✦

running the CheckM, the genome's completeness and contamination are 99.73% and 0.06%, respectively (24). These findings show that the assembled genome is of excellent quality. There was a total of 5467 Coding Sequences (CDS) predicted. 5372 of these could be assigned to a COG number that encodes proteins.

Pathogenic and intrinsic genus factors. *Achromobacter* species have intrinsic properties that allow them to survive under poor environmental conditions that would otherwise limit their dispersion. Strains of the *Achromobacter* species possess common inherent genes that help them build resistance. Our analysis

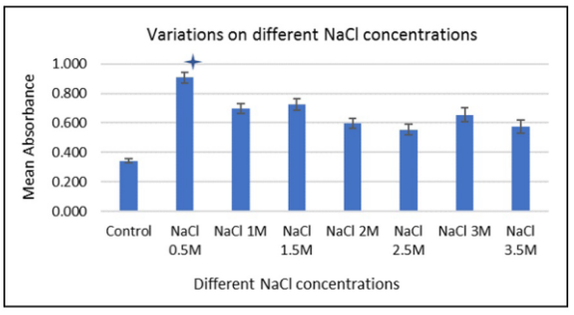


Fig. 2. Effect of different concentrations of sodium chloride supplementations on absorbance
Significant difference marked as +

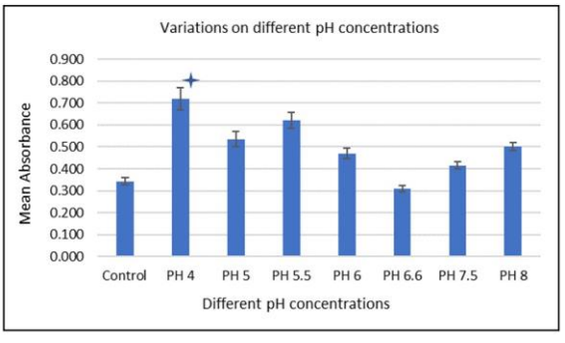


Fig. 3. Effect of different concentrations of pH on absorbance.
Significant difference marked as +

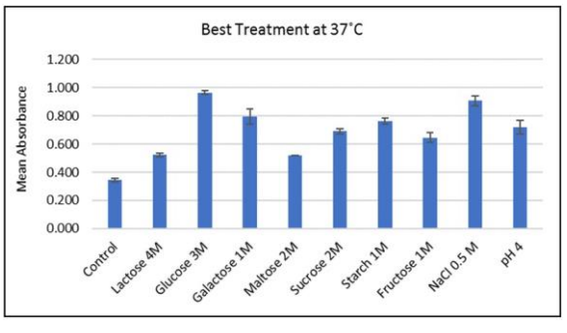


Fig. 4. Effect of best treatment at 37ºC on absorbance

identified many genes in the IA strain, which can be classified into protein secretion, potassium metabolism, DNA metabolism, protein metabolism, and denitrification categories, involved in colonization and infection. We discovered that our isolate has integrated virulence systems encompassing types I, II, VI, potassium metabolism, and denitrification that are found in other *Achromobacter* strains. A big genome rich in C-G sequences, innate resistance to arsenic and other hazardous metals, and the ability to break down aromatic chemicals are examples of such features that are common in our isolate (Table 1) (25-28).

Like other Gram-negative pathogens, exopolysaccharides are important for biosynthesis and the capsular polysaccharide ABC transporters, KpsT, KpsE, and KpsM, contribute to the formation of the extracellular matrix. Interestingly, we also found the common antimicrobial resistance genes i.e., D-beta lactamase and AxyEF-OprN efflux pump (Table 1). Multidrug resistance appears to be a prevalent characteristic in this genus, based on this finding.

DISCUSSION

Biofilm is a sessile microbial community in which cells are entwined inside a self-produced protective extracellular polymeric matrix and connected to a surface (biotic or abiotic). Recognizing that bacterial biofilm may play a role in disease pathogenesis has resulted in a greater emphasis on identifying diseases that may be biofilm-related. Because biofilm-residing bacteria can be resistant to the immune system, antibiotics, and other treatments, biofilm infections are often chronic in nature (29, 30). There are various situations where the physiology and molecular biology of a pathogen are poorly understood, emphasizing the importance of gaining better knowledge to inform the development of future treatment approaches. This study is an important step in that direction since it identifies numerous environmental and molecular variables of biofilm production that are thought to be important in *A. mucicolens* IA strain's persistence and pathogenicity.

Numerous extrinsic factors, such as the physiochemical characteristics of surface materials, temperature, nutrients, pH, salt, sugar, and the presence of other bacteria, have been shown to influence initial cell attachment and subsequent biofilm formation in addition to the intrinsic properties of individual strains (31). We investigated the production of biofilms in our isolate in media containing varying concentrations of glucose, sucrose, fructose, maltose, galactose, lactose, starch, pH, and NaCl. Although the addition of sugars and salt improved the biofilm phenotype as measured by a rise in OD, it was statistically significant. Similar results have been observed in the study performed by Yang Y et al. on the effects of sugars and antimicrobial substances on oral microbial biofilm formation (32).

Jackson et al. investigations of the effect of glucose

on *E. coli* biofilm formation found that glucose suppresses biofilm development mediated by catabolic suppression mechanisms (CRP) (33) but Roy et al., found that glucose concentration up to 0.05% glucose will increase biofilm formation on plastic (PLA) and silicon rubber (SR) by *Salmonella enterica* serotype Kentucky but after that it will inhibit viable cell growth (34). While in our study we found that an increase in glucose concentrations may inhibit biofilm production. Xu et al. investigated biofilm development in *Listeria monocytogenes, Staphylococcus aureus, Shigella boydii*, and *Salmonella* Typhimurium in different salt concentrations (10-0%). Our observation was also in accordance with Xu et al. results that showed increasing the salt concentration inhibits biofilm formation; however, a significant reduction was detected at the 2% concentration. One reason for this decrease in biofilm development is that bacterial cells in such settings have a lower hydrophobicity (35). Chai Y et al. discovered that galactose metabolism genes play a crucial role in biofilm formation and the establishment of a polysaccharide matrix in a study on the effect of galactose metabolism on the biofilm formation of *Bacillus subtilis* (EPS), which could be the effect of galactose metabolism in our strain too (36).

No research has explicitly looked at bacteria that cause respiratory illness and the effect of pH on their proliferation and the creation of a biofilm to date. However, some research has been done on the effect of pH in dental plaque biofilms and on urinary tract infections, where pH influenced biofilm growth (37, 38). Likewise, in our analysis, a pH range of 4 to 5.5 resulted in much-increased biofilm formation. These findings may point to a link between the presence of carbohydrates, NaCl, and pH in the respiratory airway and the risk of colonization or infection by pathogenic bacteria such as *Achromobacter* species.

The method of biofilm formation in *Achromobacter* species is unknown, as well as little research has been carried out on the expression profiles of genes involved in biofilm formation. In order to better understand the molecular mechanism of biofilm formation, we attempted to identify relevant genes involved in virulence and biofilm development. Our findings indicate that three genes FliF, FliM, FliN involved in flagellar motility, may be critical for adhesion. All *Achromobacter* species have peritrichous flagella, which allow for swimming motility, contribute to possible biofilm development, and may aid in host cell invasion. *Achromobacter* species, like other flagellated Gram-negative organisms, use complex membrane-bound proteins called secretion systems (types I–IV) for the extracellular trafficking of proteins and enzymes (25, 39, 40). Similarly, our IA strain genome consists of secretion systems (types I, II/IV, VI) for trafficking proteins and enzymes. Among the found genetic sequences, NarK is a crucial gene in the denitrification cluster, which has recently been reported to be important in the pathogen's interaction with its host (41). Aside from permitting anaerobic respiration, denitrification may possibly shield the organism against oxidative damage caused by the host's immunological defense (42). The inclusion of numerous denitrification genes, as well as the important Nitrate transporter NarK, contributes to our strain's pathogenesis and tolerance in adverse circumstances.

Electrical signaling has not been studied in Cystic Fibrosis pathogens to our knowledge, but potassium ion channels have been shown to increase biofilm formation and electrical signaling in biofilms of the unrelated Gram-positive Bacillus subtilis and *Acinetobacter baumannii* (43-45). Our isolate exhibits four osmosensitive K+ channel histidine kinase genes KdpD II, III, IV, and V. In addition, three genes involved in DNA recombination and repair were found in the isolate, suggesting a higher mutation frequency.

Exopolysaccharides are significant extracellular matrix constituents (46). In a biofilm, gene expression increased in genes involved in the creation and maintenance of the cell wall and capsule, as well as in the formation of the extracellular matrix. The presence of genes involved in the ABC transporter complex (KpsT/KpsM) exports polysaccharides across the cytoplasmic membrane, contributing to an increase in the production of extracellular matrix components in *Achromobacter* biofilm (47). Genes KpsT, KpsM, and KpsE presence in our strain indicate that they play a role in maintaining the integrity of the cell wall, capsule, and extracellular matrix strengthening the biofilm. Efflux pumps are important resistance mechanisms in Gram-negative bacteria, and many bacterial species have multiple RND-type multi-drug resistance pumps (29, 30, 48, 49). In *P. aeruginosa*, four operons expressing RND (resistance-nodulation-division)-type multidrug efflux pumps named MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM have overlapping but distinct substrate specificities (50, 51). The presence

of identical operons in *Achromobacter* strain AX08 was identified using RAST annotation. As a result, the AxyEF-OprN efflux pump may be involved in *A. mucicolens* IA strains' intrinsic antimicrobial resistance. In this analysis, we also found MacB in our isolate along with the MacA adaptor protein, which could influence the bacteria's infectivity. The expression of MacB-like proteins genes that are involved to expel antibiotics and exporting virulence factors influences the survival of *Salmonella* and *Streptococcus pyogenes* in macrophages (52, 53).

Every stage of biofilm growth, maintenance, and spread is controlled by a combination of environmental and genetic cues. Morgan R, et al. showed that the gene product BdlA is required for mediating the biofilm dispersion response to a variety of environmental signals, including glutamate, succinate, silver, arsenite, and mercury salts (54). The presence of the BdIA gene in our isolate, which codes for potential chemotaxis transducer protein, may play a role in the signaling cascade resulting in biofilm dispersion in *Achromobacter mucicolens.* Another gene, WspC, which is part of a potential chemosensory signal-transduction operon, has been demonstrated to cause cell aggregation and altered colony morphology in *Pseudomonas putida* (55), and our isolate. Identifying native cues in bacteria, such as BdlA or WspC genes, that are involved in sensing and transducing signals within cells, resulting in modulation of c-di-GMP levels, swimming motility, and bacterial cell surface adhesiveness is critical for understanding the subsequent stages of bacterial biofilm formation and their genetic regulation.

Because all the strains had the potential to create biofilm at various levels, but the incidence of genes was varied, the predominance of chosen genes involved in biofilm formation implies that several factors may be responsible for different steps of biofilm development. To further understand the expression of these genes in biofilm production in our *A. mucicolens* strain, more research is needed. As a result, this genetic information can be used to learn more about the pathogenicity of the bacteria, as well as the evolution of the *Achromobacter* genus.

CONCLUSION

The results show that several variables such as carbohydrate supplementation, pH, and NaCl concentrations greatly improve *A. mucicolens* biofilm-forming ability in the suggested method of in vitro biofilm formation experiment using TCP. The study outcomes are crucial in shedding light on the factors that influence the biofilm-forming capacity of *A. mucicolens,* which is an antibiotic-resistant pathogen that poses a risk to immunocompromised patients. Understanding the influence of genetic and environmental variables on biofilm formation can facilitate the development of effective treatment options for infections caused by this bacterium. The identification of genes related to virulence in the *A. mucicolens* isolate examined underscores its potential for causing persistent respiratory infections, and further investigations on gene expression could aid in comprehending the mechanisms of infection and inflammation in affected individuals. These findings hold significant implications for infection control and treatment, as they propose targeting factors that contribute to biofilm formation, such as carbohydrate supplementation, and NaCl and pH control; regulating them may be effective in limiting the spread of *A. mucicolens* infections. Moreover, identifying virulence-related genes in this bacterium may assist in developing therapies that specifically target the mechanisms of pathogenicity.

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