Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-216
Volume 106 Issue 4

Hydrophobicity and Adhesion Properties of Bacterial Growth Under Varying Temperature-Time Environment and Material Surface

Rita NN Rammo*

Department of Biology, Collage of Science, University of Kirkuk, Iraq

Abstract

Hydrophobicity and adhesion of bacterial growth under varying temperature-time environment and material surface have been studied. Bacterial isolates were identified by standard methods of classification. Hydrophobicity index was assessed by microbial adhesion to hydrocarbon (MATH) utilizing absorbance at 520 nm; and adhesion by measuring crystal violet absorbance at 570 nm for various times (1,8,24 H), temperatures (5,25,40ºC) and material surfaces (PS,PP,PTFE). The results of *P. aeruginosa* and *S. aureus* showed a strong - moderate level of hydrophobicity at 40ºC in comparison to *S. marcescens* at a low level. In the adhesion assays to plastic materials, *P. aeruginosa* and *S. aureus* showed more adherence than *S. marcescens* and following the order as Teflon<polypropylene<polystyrene for all time-temperature range studied. Our results explain that the adhesion mechanism is governed by the triad interaction bacteria environment material surface, each of which contributes its attribute that when optimum conditions are favorite lead up to infection.

Keywords: Gram +/- Bacteria; Temperature; Hydrophobicity; Adhesion; Biomaterial

Correspondence to: Rita NN Rammo, Department of Biology, Collage of Science, University of Kirkuk, Kirkuk, Iraq; Tel: 009647507670042; E-mail: rita.rammo@uokirkuk.edu.iq

Citation: Rammo RNN (2020) Hydrophobicity and Adhesion Properties of Bacterial Growth Under Varying Temperature-Time Environment and Material Surface. Prensa Med Argent, Volume 106:4. 216. DOI: https://doi.org/10.47275/0032-745X-216.

Received: February 07, 2020; **Accepted:** February 25, 2020; **Published:** March 05, 2020

Introduction

Serratia marcescens is a Gram-negative and mesophilic microorganism found in water, soil, plant, and animals; it is transmitted by direct contact and via saline solutions. Their resistive infections are normally treated with specialized formula antibiotics [1]. Now it is infamous for its pathogenicity to hospital patients but there are many reports on the contamination of medical devices and nosocomial infections with this bacterium [2]. Pseudomonas aeruginosa is a Gram negative and mesophilic strain that frequently causing nosocomial infections of patients with reduced immunity and association with infections and are usually very tough to eradicate [3]. Staphylococcus aureus is a Gram positive and mesophilic microorganism commonly associated with contamination caused by foreign body material implants as in catheter-related infection [4].

Adhesion to surfaces by bacteria and other microorganisms tend to follow a survival pattern in the colonization of surfaces [5]. Initially the growth which is a thermodynamic process is mediated by diad interactions covering physical/chemical factors which also depend on the presence of nutrient that bring about growth for the bacterial colonization [6]. Virulence factors in the bacterial adhesion depend on the physical nature of the biomaterial surface that meet the body as in the case of various implants [7,8].

Temperature is an abiotic factor that dramatically influence the efficacy of adhesion and to some extent on the hydrophobicity of bacteria / surface interface [9]. Tanaka Y, et al. (2004) reported that *S. marcescens* and other bacterial species have unusual bacterial characteristic that is, temperature- dependent to bacteriostatic activity and observed that higher environment temperatures *S. marcescens* suppress its own growth and the growth of other bacteria [10].

Material surfaces that meet microorganisms lead to the formation of biofilm by the initiation of bacterial adhesion to surfaces, a phenomenon governed by the triad interaction of physicochemical properties of the bacteria, environment and biomaterial characteristics. The main factors involved in polymeric surfaces are hydrophobicity and charge; thus negatively charged surfaces when in contact with negatively charged bacteria lead to electrostatic repulsion [11]. Most used polymers as biomaterials are polystyrene PS, polyethylene PE, polypropylene PP, polyurethane PU, polyethylene terephthalate PET, polytetrafluoroethylene PTFE (Teflon) and polymethylmethacrylate PMMA (Perspex).

As seen from the highlights the importance of these interactions as a functional bacterial growth parameters that this work is intended to investigate the influence of environmental factors (variation in growth time and growth temperature) and material surface factors on hydrophobicity and adhesion of *P. aeruginosa*, *S. marcescens* and

S. aureus cultured on different polymeric surfaces (PS, PP and PTFE).

Materials and Methods

Collection and isolation of bacteria

The bacteria isolated from clinical swaps were taken from different clinical laboratories and related hospital departments located in and around Kirkuk city to the sum of 132 swap samples. Then transferred to nutrient broth for enriching after which selected as grown on nutrient agar medium. Identification of these isolates were done biochemically [12].

Hydrophobicity assay (Microbial adhesion to hydrocarbon) MATH

The method proposed by Zabielska J, et al. (2017) with some culture modifications of *S. marcescens*, *P. aeruginosa* and *S. aureus* were activated in Luria Bertani broth (LB) by incubation for 24 hrs. at 30℃ [13]. The inoculums (A1) was mixed with P-xylene and incubated for 10 min at 5, 25 and 40℃. Samples were homogenized and inoculated once again for 45 min. The samples could be separated in two phases (aqueous phase and hydrocarbon phase). The aqueous phase (A2) was recorded by spectrophotometer at 520 nm [13]. The percentage of hydrophobicity index (HI) of cell adhesion to hydrocarbon was calculated in the following formula:

- HI (%) = [(A1 – A2) / A1] × 100

The results were evaluated according to the scale: strong hydrophobicity >50%, moderate hydrophobicty 20-50% and low hydrophobicity <20% [14].

Adhesion to biomaterials

All strains of bacteria were tested for adhesion to plastic materials includes polystyrene PS, polypropylene PP, and polytetrafluoroethylene PTFE (Teflon). Bacterial samples were cultivated in Tryptic Soy Broth (TSB) for 24 hr at 30℃. 20 µl of bacteria was carried into 230 µ of TSB on plastic plates. Plates were incubated at different periods of time (5, 25, 40℃) for 1, 8 and 24 hrs., then the content was poured out and then rinsed with sterile water. The plates were dried, and adherent bacteria cells were fixed with 96% ethanol for 20-30 min. After wards, ethanol was removed, and bacteria were stained with 0.5% crystal violet. The dye was removed, and the plates were again rinsed with water. After drying, each plate decolorized with 96% ethanol and the absorbance was measured 570 nm. The blank sample was the growth medium, categories as positive 0.1≤OD or negative 0.1> OD [15].

Statistical analysis

All tests were designed in triplicate and expressed as mean +/- standard deviation error. Results that p≤0.05 were indicated as significantly statistic.

Results and Discussion

Bacterial sample swabs isolated from the various sites were processed immediately and the percentage incidence of *S. marcescens*, *P. aeruginosa* and *S. aureus* were recorded in each sample; 15 isolates identified as *S. marcescens*, 11 as *P. aeruginosa* and 9 as *S. aureus* based on biochemical profiles and subsequent analysis. The strains selected due to their different features in order to evaluate the hydrophobicity and adhesion behavior of a Gram positive and Gram-negative microorganisms in response to changes caused by time-temperature environment and material surface.

The results of hydrophobicity index assessed by MATH method for *S. marcescens*, *P. aeruginosa* and *S. aureus* at different growth temperatures are shown in Figure 1. *S. marcescens* shows strong hydrophobicity (62%) at 5℃ and progressively decreases to moderate (38%) at 25℃ and reaches low level (17%) at 40℃ in contrast to *P. aeruginosa* and *S. aureus* that show strong level (68%) and (66%) respectively at 40℃. Thus, the mode of such moderate-strong hydrophobic properties results in colonization of abiotic surfaces. Norouzi F, et al. (2010) presented hydrophobicity data of *P. aeruginosa* assessed by MATH method and most strains demonstrated moderate hydrophobic properties [16]. Tyfa A, *et al.* (2015) tested Gram-positive *S. aureus* using the same method and found strong hydrophobic behavior [17].

Bacteria with hydrophobic properties prefer hydrophobic material surfaces, thus material hydrophobicity dominate the mechanism of adhesion of bacteria in comparison to bacterial cell hydrophobicity. The correlation between the cell hydrophobicity and adhesion to polystyrene surface under variety of growth conditions appeared that this property was not enough to predict adhesive behavior of the bacterial strains [18]. The hydrophobicity contributes to show the adhesion in different conditions by the influence of other factors, such as surface charge, presence of flagella, fimbriae and exopolysaccharide production [19].

The crystal violet binding method was used to assess *P. aeruginosa*, *S. marcescens* and *S. aureus* strains capacity of adhering to biomaterials: polystyrene, polypropylene, Teflon by which their adhesive properties were evaluated. The adhesion assay represented by the absorbance at 570 nm of the three bacteria under different growth conditions and material surfaces are displayed in figure 2 and figure 3. The results indicate that the time and temperature of the bacterial growth have varying influence on the capacity of *P. aeruginosa*, *S. marcescens* and *S. aureus* to adhere to polystyrene, polypropylene, and Teflon.

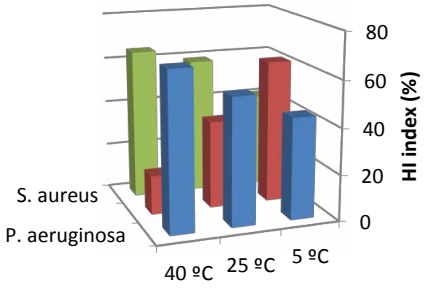

Figure 1: Influence of growth temperature on hydrophobicity index computed by the MATH method for *S. marcescens*, *P. aeruginosa* and *S. aureus*.

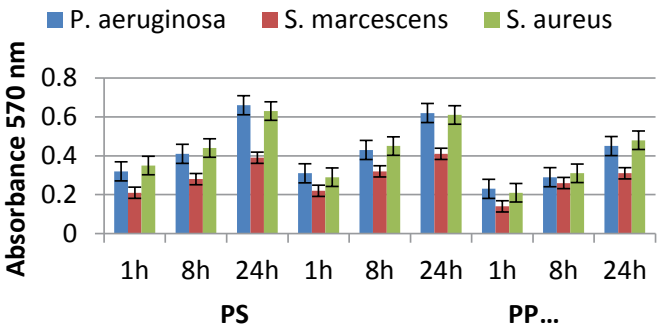

Figure 2: Variation in adhesion represented by absorbance of *P. aeruginosa*, *S. marcescens* and *S. aureus* cultured at 40℃ for different growth times (1,8,24 hrs.) on different surfaces (PS, PP, and PTFE).

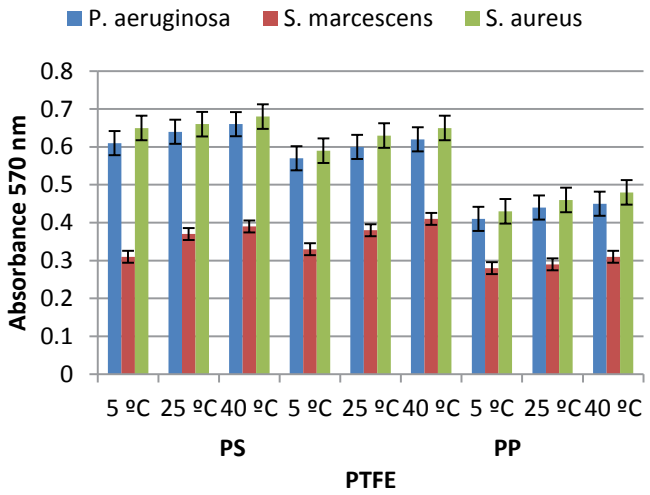

Figure 3: Influence of growth temperature (5, 25, 40°C) on adhesion of *P. aeruginosa, S. marcescens* and *S. aureus* at growth time of 24 hrs. cultured on different surfaces (PS, PP, and PTFE).

S. marcescens demonstrated to become more hydrophobic than *P. aeruginosa* and *S. aureus* for all time-temperature environment.

Regarding incubation period, all the bacterial strains were able to adhere to all plastic material surfaces after 1 hr contact with the absorbance of *P. aeruginosa* and *S. aureus* isolates in the lead. After 8 hrs. of contact for almost all strains absorbance ranged from 0.3-0.41, an indication to their moderate adherence. For long incubation period (24 hrs.) *P. aeruginosa* and *S. aureus* remained in the lead with absorbance reaching 0.66-0.68 in comparison with *S. marcescens* at 0.38. Zabielska J, *et al.* (2017) upon studying adhesion of *P. aeruginosa* on polystyrene reported similar findings [13]. Regarding incubation temperature, all *S. marcescens* strains show lowest absorbance for all temperatures indicating moderate adhesion in comparison with *P. aeruginosa* and *S. aureus* that show strong adhesion. Moderate-strong adhesion can be correlated with the reaction rate activity of enzymes and so has a bearing capacity on the development of the bacterial cells. Optimum temperature result in the healthy growth of bacterial populations; conversely, temperature away from optimum value reduce bacterial growth efficiency due to a reduction in the bacterial enzyme reaction rates [20].

Regarding material surface, the results of adhesion of the three bacteria on different surfaces are shown in Figure 4. Teflon surface show low adhesion when compared with polystyrene and polypropylene for all strains. Although Teflon show less adhesion characteristics with polystyrene and polypropylene nevertheless bacterial adhesion exhibit their own preference that depend on bacterial genus and species that dominate the adhesion mechanism. Liu Y, *et al.* (2004) have shown that the microbial adhesion strongly depends on the hydrophobic-hydrophilic properties of interacting surface [21].

Conclusion

From this work we conclude that the bacterial adhesion efficacy is dominated by the interaction of bacteria with their physical environment (temperature and contact material surface), the most important of which is the surface material where colonization occur. In addition, Teflon has shown the lowest adherence possible relative to polystyrene and polypropylene.

References

1. Gouin F, Papazian L, Martin C, Albanese J, Durbec O,et al. (1993) Non-comparative study of the efficiency and tolerance of cefepimein combination with amikacin in the treatment of severe infections in patients in intensive care. J Antimicrob Chemother 32: 205-214.https://doi.org/10.1093/jac/32.suppl_B.205
2. HejaziA,Falkiner FR (1997) *Serratia marcescens*.J Med Microbiol 46: 903-912.https://doi.org/10.1099/00222615-46-11-903
3. Breidenstein EB, de la Fuente-Núñez C, Hancock RE (2011) Pseudomonasaeruginosa: all roads lead to resistance. Trends Microbiol 19: 419-426.https://doi.org/10.1016/j.tim.2011.04.005
4. Treter J, Macedo AJ (2011) Catheters: a suitable surface for biofilm formation. Handbook: science against microbial pathogens: communicating current research and technological advances. Formatex Research Center,Spain.
5. HuntSM,Werner M,HuangB,HamiltonM,StewartPS (2004) Hypothesis for the role of nutrient starvation on biofilm detachment. Appl Environ Microbiol 70: 7418-7425. https://doi.org/10.1128/AEM.70.12.7418-7425.2004
6. DonlanRM (2002) Biofilms: microbial life on surfaces. EmInfect Dis 8: 881-889. https://dx.doi.org/10.3201/eid0809.020063
7. BusscherHJ,PoegRJ,Van der MeiHC (2009) Snapshot: biofilm and biomaterials; mechanism of medical device related infections. Biomaterials 30: 4247-4248.https://doi.org/10.1177/0022034510368644
8. FlemmingHC,Wingender J (2010) The biofilm matrix.Nat Rev Microbiol 8: 623-633. https://doi.org/10.1038/nrmicro2415
9. Cappello S, GuglielminoPP (2006) Effects of growth temperature on polystyrene adhesion of*Pseudomonas aeruginosa* ATCC27853. Brazi J Microbial 37: 205-207. https://doi.org/10.1590/S1517-83822006000300001
10. Tanaka Y, Yuasa J, Baba M, Tanikawa T, Nakagawa Y, et al. (2004) Temperature-dependent bacteriostatic activity of *S. marcescens*. Microb Environ 19: 236-240.https://doi.org/10.1264/jsme2.19.236
11. JuckerBA, HarmsH,ZehderAJB (1996) Adhesion of the positively charged bacterium *Stenotrophomanas(Xanthomonas) maltophilia* 70401 to glass and Teflon. J Bacteriol 178: 5472-5479.https://doi.org/10.1128/jb.178.18.5472-5479.1996
12. HoltJG, Krieg NR, Sneath PHA, Staley JT,WilliamsST (1994) Bergey's manual of determinative bacteriology. (9th edtn), Williams and Wilkins, United States.
13. ZabielskaJ,Kunicka-Styczynska A, Otiewska A (2017) Adhesive and hydrophobicity properties of *Pseudomonas aeruginosa* and *Pseudomonas cedrina* associated with cosmetic. Ecol Ques 28: 41-46.http://dx.doi.org/10.12775/EQ.2017.037
14. Kadam TA, Rupa L, Balhal DK, Totewad ND, Gyananath G (2009) Determination of the degree of hydro-phobicity-A technique to assess bacterial colonization on leaf surface and root region of lotus plant. Asian J Exp Sci 23: 135-139.
15. Ellafi A, Lagha R, Ben-Abdallah F, Bakhrouf A (2012) Biofilm production, adherence and hydrophobicity of starved *Shigella* in seawater. Afri J Microbiol Res 6: 4355-4359.
16. Norouzi F, Mansouri S, Moradi M, Razavi M (2010) Comparison of cell surface hydrophobicity and biofilm formation among ESBL- and non-ESBL-producing*Pseudomonas aeruginosa* clinical isolates. Afri J Microbial Res 4: 1143-1147.
17. Tyfa A, Kunicka-Styczyńska A, Zabielska J (2015) Evaluation of hydrophobicity of quantitative analysis of biofilm formation by *Alicyclobacillus* sp. Acta Biochim Pol 62: 785-790.https://doi.org/10.18388/abp.2015_1133
18. Hamadi F, Latrache H (2008) Comparison of contact angle measurement and microbial adhesion to solvents for assaying electron donor-electron acceptor (acid-base) properties of bacterial surface. Colloids Surf B 65: 134-139.https://doi.org/10.1016/j.colsurfb.2008.03.010
19. Zeraik AE, Nitschke M (2012) Influence of growth media and temperature on bacterial adhesion to polystyrene surfaces. Braz Arch Biol Technol 55: 569-576.
20. Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Pro Nat Sci 18: 1049-1056.https://doi.org/10.1016/j.pnsc.2008.04.001
21. Liu Y, Yang SF, Li Y, Xu H, Qin L, et al. (2004) The influence of cell and substratumsurface hydrophobicities on microbial attachment.J Biotechnol 110: 251-256.https://doi.org/10.1016/j.jbiotec.2004.02.012