JKAU: Mar. Sci., **Vol. 31** No. 2 pp: 45-54 (1442 A.H. / 2021 A.D.)
DOI :10.4197/Mar.31-2.4

Isolation and Identification of Biofilm Bacteria from Microfouling Assemblage Developed on Artificial Materials Submerged in the Red Sea

Idris Abdulrahman[1]*, Mamdoh T. Jamal[1], Majed Alshaery[2], Saleh M. Al-maaqar[2,3] and **Sathianeson Satheesh[1]**

[1]Department of Marine Biology, Faculty of Marine Sciences, King Abdulaziz University, P. O. Box 80207 Jeddah 21589, [2]Department of Biological Sciences, Faculty of Science, King Abdulaziz University Jeddah, Saudi Arabia, and [3]Department of Biology, Faculty of Education, Al-Baydha University, Al-Baydha, Yemen

* iabdulrahman@stu.kau.edu.sa

Abstract. Biofilm bacteria are primary surface colonizers in marine biofouling assemblage on submerged surfaces and dominate the early microfouling phase. They are an important target in the design of antifouling treatment due to their ability to initiate biofouling and support of the subsequent macrofouling colonization. In this study, several biofilm bacteria were isolated from Petri dish and polyvinyl chloride (PVC) pipes submerged in the Red Sea coast for a week. The biofilm-forming bacteria were isolated by spread plate method under standard conditions and identified by 16S rRNA sequencing method. Each of the isolates was evaluated for biofilm formation qualitatively using the tube assay. Microtiter plate assay was used to quantify the biofilm produced by the selected organisms. A total of 11 out of 21 isolated bacteria were able to form biofilm under laboratory conditions. Most of the isolates (7 out of 11) are from the genus *Pseudoalteromonas* and one isolate each from the genera *Halomonas*, *Marinomonas*, *Psychrobacter* and *Vibrio*. This study indicated that the bacterial community forming the biofilms on hard substrates in the Red Sea are diverse and capable of forming biofilms on surfaces under laboratory conditions. These isolates could be used as target microorganisms for antifouling screening assays.

Keywords: Biofilm; bacteria; biofouling; antifouling; *Pseudoalteromonas*; Red Sea.

1. Introduction

Marine biofouling occurs on all submerged surfaces following colonization by living organisms in marine environment. It is an undesirable process that has economic and environmental consequences (Cruz, 2020). It is impacting negatively on the finances of maritime, naval, tourism, aquaculture, and fisheries industries due to operational and maintenance cost to prevent the attachment of the organisms through the application of protective coatings or in their labour-intensive removal and increase in fuel consumption (Mathew *et al*., 2021). To the environment, fuel increase causes increase burning of greenhouse gases for hull and propeller machines and several coatings applied to prevent biofouling causes water pollution and alters the marine ecological setup (Farkas *et al*., 2021). The consequence of biofouling is increase in transportation cost. It also affects other services such as service delivery and quality of the products.

Biofouling is a sequential process that involves the attachment of different organisms at different stages at distinct time interval. It begins with the conditioning of the surface by organic matter followed by development of microfouling assemblage dominated by extracellular polymeric substances (EPS)

producing bacteria and diatoms. The microfouling phase marks the initial stage of the colonization process by living organisms leading to microbial biofouling (biofilm) formation (Grasland *et al*., 2003). The biofilm communities colonize the surfaces rapidly in the multistage process less than one hour of the surface contact with water to alter its chemistry due to their attachment strength (Salta *et al*., 2013). Bacteria dominate in the biofilm among surface associated microbes with its high rate of occurrence by attaching to the surface faster than other organisms (Zobell and Allen, 1935; Papadatou *et al*., 2021). They colonize both living and non-living surfaces and the biofilm is held firmly by EPS secreted by the bacteria that enable their attachment and modification of the submerged surface (Renner and Weibel, 2011). This modification enables the biofilm bacteria to colonize surfaces quickly and further provide access for the subsequent colonization by macrofouling organisms (Lorite *et al*., 2011). The EPS form a matrix to hold the individual organisms together which allow the bacteria to withstand harsh environmental conditions (Flemming *et al*., 2007). The biofilm is a highly dynamic, stable, heterogeneous structure that can survive for longer period (Flemming, 2008; Salta *et al*., 2013). The structure is difficult to remove by cleaning when it matures on surfaces (Flemming, 2008) and is affected by site and type of substrates (Briand *et al*., 2012). Despite protective coatings applied on surfaces to prevent attachment of fouling organisms, microfouling assemblage still occurs due to adhesion strength of the biofilm forming organisms (Oliveira and Granhag, 2016). This stage can have impact on the performance of ships as such, it is important in determining the efficacy of antifouling treatments (Hearin *et al*., 2016). To improve the effectiveness of antifouling strategies, the strength, nutritional requirement, and physico-chemical properties of the biofilm bacteria is very relevant.

Different biofilm bacteria are involved in the assemblage making them an important target in preventing and controlling biofouling. The composition of the bacterial communities' changes with time and seasonal variation making the communities highly variable and complex (Sawant *et al*., 1995). All bacterial phyla are involved but proteobacteria often dominates the stage which can be attributed to their success in the competition for space and nutrients during the biofilm development stages and production of inhibitory substances (Burchard and Sorongon, 1998; Matz *et al*., 2004). The biofilm influences the metamorphosis of the larvae of benthic invertebrates (Hadfield, 2010) and release of macroalgal spores (Goecke *et al*., 2010). The larval settlement is dictated by the nature and type of the biofilm communities (Dobretsov and Qian, 2006) which serve as natural settlement cues for the larvae to choose the right settlement site (Qian *et al*., 2007). The settlement is also enhanced or inhibited by the properties of the bacteria forming the biofilm in the environment (Dobretsov *et al*., 2006).

Understanding the early stage of biofouling (microfouling) is important for the development of non-toxic techniques to control biofouling on surfaces (Qian *et al*., 2007). This depends on the identification of the different biofilm bacteria involved and their physico-chemical properties (Grasland *et al*., 2003; Afonso *et al*., 2021). Microfouling assemblage can be estimated based on the bacterial cell density forming the biofilm and relate directly with the rate of attachment of subsequent colonizers (Dang and Lovell, 2015). Biofilm is detrimental to all submerged surface in seawater. Understanding the diversity of the biofilm bacteria which are the dominant cause of microfouling assemblage and factors relevant in biofilm formation are important in combating biofouling. This is an important consideration in antifouling technologies to minimize or prevent the problem and it completes eradication.

In this study, biofilm bacteria from two substrates (Petri dish and PVC pipes) submerged in the Red Sea coast of Central Red

Sea were isolated and identified with morphological and 16S rRNA features. This will have significant contribution in the development of control strategies against the occurrence of microfouling on submerged surfaces in marine environments.

2. Materials and Method

2.1 Isolation and Identification of Microfouling Bacteria

The formation of microfouling communities was studied by submerging polyvinyl chloride (PVC) pipes and sterile Petri dishes (SaudiPlast, Saudi Arabia) at 2m depth in the Obhur creek of the Red Sea (N21°42.562′ E39°05.764′) in six replicates each. The PVC pipes are white in colour, cut to 15cm each per piece in length and a rope was tight to one end of the pipe and then submerged in the sea by hanging the rope tightened to the Jetty stationed at Obhur creek. The petri dish which is made up of polystyrene is 90 mm in diameter. A hole is made at a side of the plate to hang the rope before submerging it in the sea in the same way as PVC. This was carried out at the beginning of fall in September 2021. The method of Balqadi *et al*. (2018) was adopted and modified for the development of biofilm on the submerged surfaces. In brief, prior to submersion in water, the PVCs and Petri dishes were cleaned, dried, and sterilized with 99 % ethanol. They were allowed to stay for at least 48 hours after exposure in the sea water after which they were removed, rinsed in sterilized filtered sea water (SFSW) and immersed in a sterilized container containing SFSW before transporting it to the laboratory. At the laboratory, microfouling bacteria were isolated from the microfouling assemblage using traditional culture method with Zobell marine agar (ZMA) after vigorous agitation of the container to release the attached biofilm bacteria. Serial dilution was carried out using SFSW using ten-fold dilution, inoculated in ZMA and incubated at 28 ºC for 48 hours. Individual distinct colonies were subcultured on fresh ZMA plates and incubated as before. Unique organisms identified based on colony morphology; microscopic features were screened for biofilm formation ability. Morphologically distinct bacterial strains were selected, re-streaked, purified and tested for its biofilm forming ability through crystal violet assay.

2.2 Assessment of Biofilm Formation Ability

To assess the ability of the strains to form biofilm, crystal violet (CV) assay described by Haney *et al*. (2018) was followed with some modifications. Briefly, to prepare the inoculum, one colony of each of the bacterial isolate was inoculated in 10 ml Zobell marine broth (ZMB) and incubated with shaking at 150 rpm overnight at 28 ºC. Fresh overnight culture of each of the organism (10%) was inoculated in borosilicate glass tubes containing 5ml of ZMB and incubated for 24 hours (incubated for 12 hours with shaking in a shaker incubator at 150 rpm and 12 hours under static condition) at 28 ºC. The planktonic cells were removed gently, and the tubes were washed with phosphate buffered saline (PBS) twice and allowed to dry. To the attached cells, crystal violet at 0.2% was added and the tubes were incubated for 20 mins at room temperature without shaking. The tubes were then washed again with PBS after discarding the CV as described before and allowed to dry. The biofilm formation was observed directly with the eyes and in most cases a visible coloured ring formed at the interface in the tube or at the bottom is interpreted as a sign of biofilm formation.

2.3 Microtiter Biofilm Quantification

The biofilm bacteria screened were subjected further for biofilm quantification using the microtiter quantification assay. The procedure of O'Toole (2011) was adopted and modified. In brief, the 96-well round bottom microplates previously sterilized with 95% ethanol were inoculated with 200 µl of autoclaved FSW for 1 h prior to condition the wells. Overnight culture of each biofilm bacteria was diluted in fresh media (1:100) and 200 µl aliquot was suspended into the microtiter wells

(n=12). Control contains 200 µl media only without bacterial culture to detect contamination. The plates were incubated statically for 48 h at 28 °C. The planktonic suspension was carefully removed with a multichannel pipette into a new 96 well plates. The absorbance was read at an optical density of 570 nm in a microplate reader. For the attached cells in the plates, 300 µl of PBS was suspended into each well using a multichannel pipette to wash the plates. This was repeated 3 times and dried in inverted position. The attached cells were stained using 200 µl of 0.2% crystal violet (CV) in water and incubated at room temperature for 20 minutes after which the excess CV stain was removed by washing the plates twice as described above. The leftover of the CV was solubilized with 200 µL of 96 % ethanol to destain the wells and incubated with shaking for 15 min at room temperature and then transferred to fresh 96 well plates. The plates were read with the microplate reader (Synergy, Biotek) at 570 nm (OD_{570}). Biofilm formation ability was recorded as highly positive, low grade positive or negative when OD_{570} is ≥ 1, 0.1 to < 1 or < 0.1 respectively (Lagha *et al*., 2019).

2.4 Identification of Biofilm Bacteria by 16s rRNA Method

The promising biofilm bacterial strains were then identified up to species level through 16S rRNA gene sequencing. Genomic DNA was extracted from each of the isolate and polymerase chain reaction (PCR) was carried to amplify the 16S rRNA gene sequence with universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (AAGGAGGTGATCCACCC). The 16S rRNA gene was sequenced at Macrogen inc. and sequences obtained were analyzed and compared with closely related sequences available in NCBI using BLAST limiting the search to sequences from type materials (Zhang *et al*., 2000). The processed sequences were submitted to NCBI GenBank and their respective accession numbers were obtained. This was followed by phylogenetic tree construction using MEGA neighbour joining method to determine the phylogenetic position of the strains and their evolutionary relatedness (Kumar *et al*., 2018).

3. Results

3.1 Identification and Confirmation of Biofilm Bacteria

A total of 11 strains of microfouling bacteria isolated from the PVCs and Petri plates were identified and confirmed as biofilm bacteria from 21 different isolates. The biofilm forming strains were labelled as IMB1, IMB2, IMB8, IMB10, IMB11 IMB12, IMB13, IMB14, IMB15, IMB16 and IMB17. All the strains were Gram-negative and have mucoid colonies except IMB2 and IMB8. From PVC, IMB12 - 17 were confirmed to be biofilm-forming bacteria while IMB 1, IMB2, IMB8, IMB10 and IMB 11 isolated from Petri dish plates were having the ability for biofilm formation. Based on 16S rRNA gene sequences and similarity search, 7 of the 11 microfouling bacteria isolated were identified as members of the *Pseudoalteromonas* (IMB1, IMB10, IMB12, IMB13, IMB14, IMB15, and IMB17). The other bacterial strains identified include *Halomonas* (IMB2), *Psychrophile* (IMB8), *Vibrio* (IMB11) and *Marinomonas* (IMB16) genera. They all belong to the proteobacteria phylum and all except *Marinomonas* (an alphaproteobacteria) are gammaproteobacteria. Details of each isolate with the closest strain are provided in Table 1. Based on phylogenetic relatedness, all the isolates are closely related to each other as shown in Fig. 1.

3.2 Biofilm Quantification

The result of biofilm quantification is shown in Fig. 2. The value which represents an average of 12 replicates ranges from 0.36 to 1.57. The categorization of the biofilm bacteria as low grade positive and highly positive is presented in Table 2 as quantified based on the OD_{570} absorbance values. Apart from two isolates that were classified as highly positive, all the remaining 9 isolates are low grade biofilm formers.

Table 1. Identification of biofilm-forming bacteria isolated from microfouling assemblage formed on PVC pipes and Petri dish.

Isolates	Substrate	Identification (closest NCBI relative)	Gram reaction
IMB1	**Petri Dish**	*Pseudoalteromonas sp.* (97.93%)	Gram negative
IMB2	**Petri Dish**	*Halomonas sp.* (81.54%)	Gram negative
IMB8	**Petri Dish**	*Psychrobacter sp.* (97.16%)	Gram negative
IMB10	**Petri Dish**	*Pseudoalteromonas sp.* (95.36%)	Gram negative
IMB11	**Petri Dish**	*Vibrio alginolyticus* (96.67%)	Gram negative
IMB12	**PVC**	*Pseudoalteromonas issachenkonii* (97.73%)	Gram negative
IMB13	**PVC**	*Pseudoalteromonas shioyasakiensis* (97.67%)	Gram negative
IMB14	**PVC**	*Pseudoalteromonas gelatinilytica* (97.59%)	Gram negative
IMB15	**PVC**	*Pseudoalteromonas gelatinilytica* (99.01%)	Gram negative
IMB16	**PVC**	*Marinomonas aquiplantarum* (96.71%)	Gram negative
IMB17	**PVC**	*Pseudoalteromonas sp.* (97.26%)	Gram negative

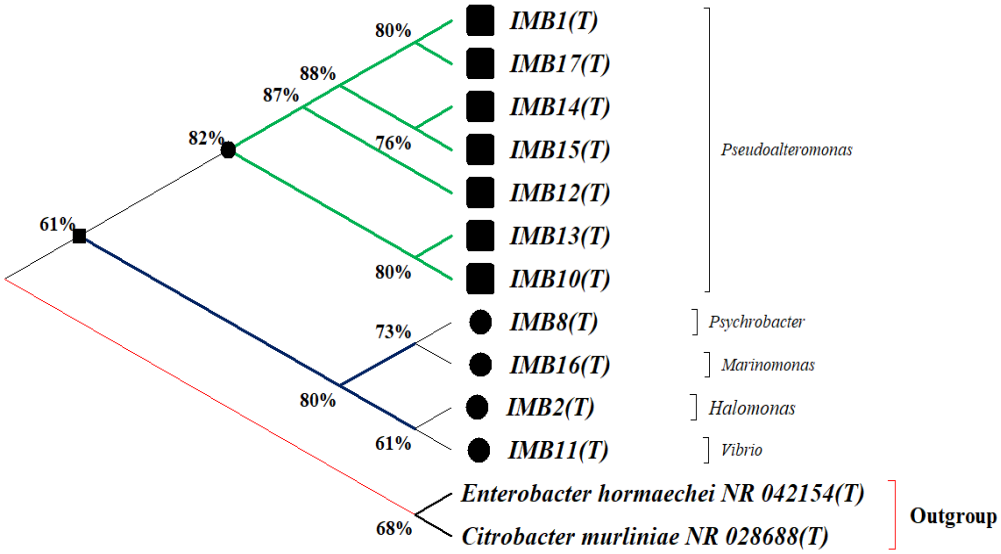

Fig. 1. Phylogenetic tree showing evolutionary relationship among the biofilm bacteria identified from submerged substrates of PVC and Petri dish.

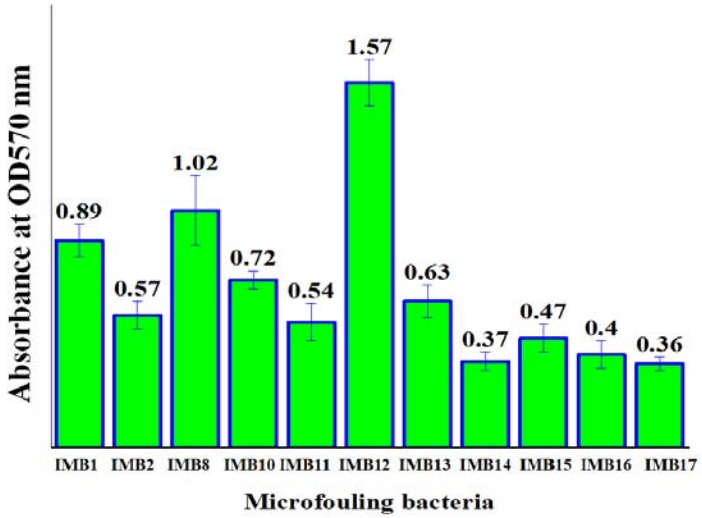

Fig. 2. Biofilm quantification of microfouling bacteria isolated from submerged PVC pipes and Petri dish at OD_{570}. Results presented is an average of 12 replicates with standard deviation.

Table 2. Categorization of biofilm formation by biofilm bacteria isolated from submerged PVC pipes and Petri dish.

Isolate code	Substrate	Biofilm categorization
IMB1	**Petri Dish**	low-grade positive
IMB2	**Petri Dish**	low-grade positive
IMB8	**Petri Dish**	Highly positive
IMB10	**Petri Dish**	low-grade positive
IMB11	**Petri Dish**	low-grade positive
IMB12	**PVC**	Highly positive
IMB13	**PVC**	low-grade positive
IMB14	**PVC**	low-grade positive
IMB15	**PVC**	low-grade positive
IMB16	**PVC**	low-grade positive
IMB17	**PVC**	low-grade positive

4. Discussion

Biofilm formation by bacteria is a necessary requirement for the development of microfouling assemblage on surfaces. The control of biofilm formation by bacteria has great potential in the prevention or minimization of biofouling on surfaces (Flemming, 2011). In the biofilm development, different surfaces attract diverse bacteria (Kerr *et al*., 1998) because the surface properties have effect on the development of microfouling (Dobretsov *et al*., 2013). Diverse bacteria are involved in biofilm formation on submerged surfaces during initial biofouling forming stage, the microfouling stage (Chen *et al*., 2013). In this study, we used two different surfaces to study the diversity of biofilm bacteria capable of biofilm formation. A diverse group of biofilm bacteria were reported in this study from the PVC and Petri Dish surfaces with the genus *Pseudoalteromonas* dominating among all the genera identified. The bacteria isolated from PVC are less diverse than those isolated from Petri dish. All of the isolates from PVCs are members of the genus *Pseudoalteromonas* except one organism that belong to the genus *Marinomonas*. The Petri dish comprises of four different genera from 5 bacterial isolates which are *Vibrio, Halomonas, Psychrobacter* and *Pseudoalteromonas*. The difference in species composition may be due to the composition and colour of the substrates used in this study. The Petri dish is made up of polystyrene and is transparent while the PVC is made up of polyvinyl chloride and white in colour. These differences can affect the attachment of the different biofilm bacteria due to different properties and structure of the surface (Camps *et al*., 2014). Colour of the substrate is important in determining the attachment and colonization of surfaces by biofilm bacteria (Dobretsov *et al*., 2013; Balqadi *et al*., 2018). Surfaces that differ in hydrophobicity, hydrophilicity, roughness, or topography will be colonized differently (Kim *et al*., 2022). Different biofilm bacteria will attach differently on the same surfaces based on their lifestyle due to their properties such as motility, cell-cell communication (Vance, 2019; Zheng *et al*., 2021). This is an indication on how the type and nature of surfaces dictate the type of biofilm bacteria that will colonize the surfaces and its diversity.

The bacterial biofilm is an important target of antifouling compounds, and their diversity is relevant in the design and implementation of antifouling technologies (Qian *et al*., 2007). Attachment to surfaces by biofilm bacteria determines the success of the initial colonization process and subsequent colonizations (Slightom and Buchan, 2009). Once the diverse bacteria colonize the surfaces, they accumulate forming films on surfaces which aggregate and begin the process of the microfouling. The microfouling assemblage is held firmly by a matrix of EPS which comprises of majorly polysaccharides,

proteins, as slimy layer which allow the biofilm bacteria to colonize many surfaces. The bacterial cells are closely associated with each other in high densities (Sutherland, 1999). Other factors that support the aggregation of the biofilm structure, shape and functions comprises of intra and inter species interaction among the species and ability to survive the competition (Dang and Lovell, 2015).

The domination of *Pseudoalteromonas* is reflected on its ability to outcompete other species in the biofilm formation process. *Pseudoalteromonas* is well known to be significant biofilm forming bacteria in biofouling process for it out competition of other species in biofilm communities and in inducing metamorphosis and settlement of marine invertebrates (Bowman, 2007). The organism is known to grow rapidly and form biofilm at a faster rate couple with the production of inhibitory compounds against other bacteria (Rao *et al*., 2005). This gives the organism greater advantage than other bacteria forming the multispecies biofilm on surfaces in natural biofilm formation (Rao *et al*., 2010). Multispecies biofilm is evidence of inter/intra specific association among the bacteria with benefits or inhibition through their metabolic products and it's the survival of the fittest through competition (Guillonneau *et al*., 2018).

The biofilm formation observed in the tubes and in the microtiter plates is an indication of the strains ability to form biofilm. Formation of biofilm in a ring form on the side or bottom of the well in the tubes via the CV stain is an evidence of biofilm formation (O'Toole, 2011). The evidence of biofilm formation is necessary to determine the ability to form microfouling assemblage by the bacteria. Members of the genera identified in this study *Halomonas Marinomonas, vibrio, Psychrobacter* and *Pseudoalteromonas* were reported to produce biofilm on surfaces (Heyrman *et al*., 2002; Rao *et al*., 2010; Bernbom *et al*., 2013; Brian-Jaisson *et al*., 2016; Balqadi *et al*., 2018; Aykin *et al*., 2019; Delacuvellerie *et al*., 2021). While biofilm formation gives the bacteria the ability to overcome many stresses in the marine environment, but its deleterious effect on surfaces leading to microfouling development causes damage to marine structures and installations (de Carvalho, 2018).

5. Conclusion

In conclusion, several biofilm bacteria from different genera are involved in the formation of microfouling assemblage on surfaces through their ability to form biofilm structure. The organisms form multispecies biofilm and most of the biofilm bacteria are Gram-negative bacteria. The organisms are important consideration in antifouling studies and can be good candidates for used as target in antibiofilm assays.

References

Afonso, A. C., Gomes, I. B., Saavedra, M. J., Giaouris, E., Simoes, L. C. and **Simoes, M.** (2021). Bacterial coaggregation in aquatic systems. *Water Research,* **196**. doi:10.1016/j.watres.2021.117037

Aykin, E., Omuzbuken, B. and **Kacar, A.** (2019). Microfouling bacteria and the use of enzymes in eco-friendly antifouling technology. *Journal of Coatings Technology and Research,* **16**(3): 847-856. doi:10.1007/s11998-018-00161-7

Balqadi, A. A., Salama, A. J. and **Satheesh, S.** (2018). Microfouling development on artificial substrates deployed in the central Red Sea. *Oceanologia,* **60**(2): 219-231. doi:https://doi.org/10.1016/j.oceano.2017.10.006

Bernbom, N., Ng, Y. Y., Olsen, S. M. and **Gram, L.** (2013). Pseudoalteromonas spp. serve as initial bacterial attractants in mesocosms of coastal waters but have subsequent antifouling capacity in mesocosms and when embedded in paint. *Applied and Environmental Microbiology,* **79**(22): 6885-6893. doi:10.1128/AEM.01987-13

Bowman, J. P. (2007). Bioactive Compound Synthetic Capacity and Ecological Significance of Marine Bacterial Genus Pseudoalteromonas. *Marine Drugs,* **5**(4): 220-241.

Brian-Jaisson, F., Molmeret, M., Fahs, A., Guentas-Dombrowsky, L., Culioli, G., Blache, Y., . . . Ortalo-Magné, A. (2016). Characterization and anti-biofilm activity of extracellular polymeric substances produced by the marine biofilm-forming bacterium Pseudoalteromonas ulvae strain TC14. *Biofouling,* **32**(5): 547-560. doi:10.1080/08927014.2016.1164845

Briand, J.-F., Djeridi, I., Jamet, D., Coupé, S., Bressy, C., Molmeret, M., . . . Blache, Y. (2012). Pioneer marine

biofilms on artificial surfaces including antifouling coatings immersed in two contrasting French Mediterranean coast sites. *Biofouling,* **28**(5): 453-463. doi:10.1080/08927014.2012.688957

Burchard, R. P. and **Sorongon, M. L.** (1998). A gliding bacterium strain inhibits adhesion and motility of another gliding bacterium strain in a marine biofilm. *Appl Environ Microbiol,* **64**(10): 4079-4083. doi:10.1128/aem.64.10.4079-4083.1998

Camps, M., Barani, A., Gregori, G., Bouchez, A., Berre, B. L., Bressy, C., . . . Lovell, C. R. (2014). Antifouling Coatings Influence both Abundance and Community Structure of Colonizing Biofilms: a Case Study in the Northwestern Mediterranean Sea. *Applied and Environmental Microbiology, 80*(16): 4821-4831. doi:doi:10.1128/AEM.00948-14

Chen, C.-L., Maki, J. S., Rittschof, D. and **Teo, S. L.-M.** (2013). Early marine bacterial biofilm on a copper-based antifouling paint. *International Biodeterioration and Biodegradation, 83*, 71-76. doi:https://doi.org/10.1016/j.ibiod.2013.04.012

Cruz, H. (2020). *Biofouling Challenges and Possible Solutions, 1.20.003 – Final Project Report.* Retrieved from Launceston, Australia.

Dang, H. and **Lovell, C. R.** (2015). Microbial Surface Colonization and Biofilm Development in Marine Environments. *Microbiology and molecular biology reviews : MMBR,* **80**(1): 91-138. doi:10.1128/MMBR.00037-15

de Carvalho, C. C. (2018). Marine Biofilms: A Successful Microbial Strategy With Economic Implications. *Frontiers in Marine Science,* **5**: 126.

Delacuvellerie, A., Benali, S., Cyriaque, V., Moins, S., Raquez, J.-M., Gobert, S. and **Wattiez, R.** (2021). Microbial biofilm composition and polymer degradation of compostable and non-compostable plastics immersed in the marine environment. *Journal of Hazardous Materials,* **419**: 126526. doi:https://doi.org/10.1016/j.jhazmat.2021.126526

Dobretsov, S., Dahms, H.-U. and **Qian, P.-Y.** (2006). Inhibition of biofouling by marine microorganisms and their metabolites. *Biofouling,* **22**(1): 43-54. doi:10.1080/08927010500504784

Dobretsov, S. and **Qian, P.-Y.** (2006). Facilitation and inhibition of larval attachment of the bryozoan Bugula neritina in association with mono-species and multi-species biofilms. *Journal of Experimental Marine Biology and Ecology,* **333**(2): 263-274. doi:https://doi.org/10.1016/j.jembe.2006.01.019

Dobretsov, S., Abed, R. M. M. and **Voolstra, C. R.** (2013). The effect of surface colour on the formation of marine micro and macrofouling communities AU - Dobretsov, Sergey. *Biofouling,* **29**(6): 617-627. doi:10.1080/08927014.2013.784279

Farkas, A., Degiuli, N. and **Martić, I.** (2021). The impact of biofouling on the propeller performance. *Ocean Engineering,* **219**: 108376. doi:https://doi.org/10.1016/j.oceaneng.2020.108376

Flemming, H.-C., Neu, T. R. and **Wozniak, D. J.** (2007). The EPS matrix: the "house of biofilm cells". *Journal of Bacteriology,* **189**(22): 7945-7947. doi:10.1128/JB.00858-07

Flemming, H.-C. (2008). Why Microorganisms Live in Biofilms and the Problem of Biofouling. In *Springer Series on Biofilms* (pp. 1-10). Berlin, Heidelberg: Springer Berlin Heidelberg.

Flemming, H.-C. (2011). Microbial Biofouling: Unsolved Problems, Insufficient Approaches, and Possible Solutions. In H.-C. Flemming, J. Wingender, and U. Szewzyk (Eds.), *Biofilm Highlights* (pp. 81-109). Berlin, Heidelberg: Springer Berlin Heidelberg.

Goecke, F., Labes, A., Wiese, J. and **Imhoff, J. F.** (2010). Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series,* **409**: 267-299. doi:https://doi.org/10.3354/meps08607

Grasland, B., Mitalane, J., Briandet, R., Quemener, E., Meylheuc, T., Linossier, I., . . . Haras, D. (2003). Bacterial biofilm in seawater: Cell surface properties of early-attached marine bacteria. *Biofouling,* **19**(5): 307-313. doi:10.1080/0892701031000121041

Guillonneau, R., Baraquet, C., Bazire, A. and **Molmeret, M.** (2018). Multispecies Biofilm Development of Marine Bacteria Implies Complex Relationships Through Competition and Synergy and Modification of Matrix Components. *Frontiers in Microbiology,* **9**. doi:10.3389/fmicb.2018.01960

Hadfield, M. G. (2010). Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. *Annual Review of Marine Science,* **3**(1): 453-470. doi:10.1146/annurev-marine-120709-142753

Haney, E. F., Trimble, M. J., Cheng, J. T., Vallé, Q. and **Hancock, R. E. W.** (2018). Critical Assessment of Methods to Quantify Biofilm Growth and Evaluate Antibiofilm Activity of Host Defence Peptides. *Biomolecules,* **8**(2): 29. doi:10.3390/biom8020029

Hearin, J., Hunsucker, K. Z., Swain, G., Gardner, H., Stephens, A. and **Lieberman, K.** (2016). Analysis of mechanical grooming at various frequencies on a large scale test panel coated with a fouling-release coating. *Biofouling,* **32**(5): 561-569. doi:10.1080/08927014.2016.1167880

Heyrman, J., Balcaen, A., De Vos, P. and **Swings, J.** (2002). Halomonas muralis sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). *Int J Syst Evol Microbiol,* **52**(Pt 6): 2049-2054. doi:10.1099/00207713-52-6-2049

Kerr, A., Cowling, M. J., Beveridge, C. M., Smith, M. J., Parr, A. C. S., Head, R. M., . . . Hodgkiess, T. (1998). The early stages of marine biofouling and its effect on two types of optical sensors. *Environment International,* **24**(3): 331-343. doi:https://doi.org/10.1016/S0160-4120(98)00011-7

Kim, T., Kwon, S., Lee, J., Lee, J. S. and **Kang, S.** (2022). A metallic anti-biofouling surface with a hierarchical

topography containing nanostructures on curved micro-riblets. *Microsystems and Nanoengineering,* **8**(1): 6, doi:10.1038/s41378-021-00341-3

Kumar, S., Stecher, G., Li, M., Knyaz, C. and **Tamura, K.** (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution,* **35**(6): 1547-1549.

Lagha, R., Ben Abdallah, F., AL-Sarhan, B. O. and **Al-Sodany, Y.** (2019). Antibacterial and Biofilm Inhibitory Activity of Medicinal Plant Essential Oils Against Escherichia coli Isolated from UTI Patients. *Molecules,* **24**(6): 1161.

Lorite, G. S., Rodrigues, C. M., de Souza, A. A., Kranz, C., Mizaikoff, B. and **Cotta, M. A.** (2011). The role of conditioning film formation and surface chemical changes on Xylella fastidiosa adhesion and biofilm evolution. *Journal of Colloid and Interface Science,* **359**(1): 289-295. doi:https://doi.org/10.1016/j.jcis.2011.03.066

Mathew, N. T., Kronholm, J., Bertilsson, K., Despeisse, M. and **Johansson, B.** (2021). Environmental and Economic Impacts of Biofouling on Marine and Coastal Heat Exchangers. In Y. Kishita, M. Matsumoto, M. Inoue, and S. Fukushige (Eds.), *EcoDesign and Sustainability II: Social Perspectives and Sustainability Assessment* (pp. 385-398). Singapore: Springer Singapore.

Matz, C., Bergfeld, T., Rice, S. A. and **Kjelleberg, S.** (2004). Microcolonies, quorum sensing and cytotoxicity determine the survival of Pseudomonas aeruginosa biofilms exposed to protozoan grazing. *Environ Microbiol,* **6**(3): 218-226. doi:10.1111/j.1462-2920.2004.00556.x

O'Toole, G. A. (2011). Microtiter dish biofilm formation assay. *JoVE (Journal of Visualized Experiments)*(47), e2437.

Oliveira, D. and **Granhag, L.** (2016). Matching Forces Applied in Underwater Hull Cleaning with Adhesion Strength of Marine Organisms. *Journal of marine science and engineering,* **4**(4): 66.

Papadatou, M., Robson, S. C., Dobretsov, S., Watts, J. E. M., Longyear, J. and **Salta, M.** (2021). Marine biofilms on different fouling control coating types reveal differences in microbial community composition and abundance. *MicrobiologyOpen,* **10**(4): e1231-e1231. doi:10.1002/mbo3.1231

Qian, P. Y., Lau, S. C. K., Dahms, H. U., Dobretsov, S. and **Harder, T.** (2007). Marine Biofilms as Mediators of Colonization by Marine Macroorganisms: Implications for Antifouling and Aquaculture. *Marine Biotechnology,* **9**(4): 399-410. doi:10.1007/s10126-007-9001-9

Rao, D., Webb, J. S. and **Kjelleberg, S.** (2005). Competitive interactions in mixed-species biofilms containing the marine bacterium Pseudoalteromonas tunicata. *Applied and Environmental Microbiology,* **71**(4): 1729-1736. doi:10.1128/AEM.71.4.1729-1736.2005

Rao, D., Webb, J. S. and **Kjelleberg, S.** (2006). Microbial colonization and competition on the marine alga Ulva australis. *Appl Environ Microbiol,* **72**(8): 5547-5555. doi:10.1128/aem.00449-06

Rao, D., Skovhus, T., Tujula, N., Holmström, C., Dahllöf, I., Webb, J. S. and **Kjelleberg, S.** (2010). Ability of Pseudoalteromonas tunicata to colonize natural biofilms and its effect on microbial community structure. *FEMS Microbiol Ecol,* **73**(3): 450-457. doi:10.1111/j.1574-6941.2010.00917.x

Renner, L. D. and **Weibel, D. B.** (2011). Physicochemical regulation of biofilm formation. *MRS Bull,* **36**(5): 347-355. doi:10.1557/mrs.2011.65

Salta, M., Wharton, J. A., Blache, Y., Stokes, K. R. and **Briand, J. F.** (2013). Marine biofilms on artificial surfaces: structure and dynamics. *Environ Microbiol,* **15**(11): 2879-2893. doi:10.1111/1462-2920.12186

Sawant, S. S., Khandeparker, D., Tulaskar, A., Venkat, K. and **Garg, A.** (1995). Corrosion and microfouling of copper and its alloys in a tropical marine waters of India (Mangalore). *Indian Journal of Chemical Technology,* **2**(6): 322-326.

Slightom, R. N. and **Buchan, A.** (2009). Surface Colonization by Marine Roseobacters: Integrating Genotype and Phenotype. *Applied and Environmental Microbiology,* **75**(19): 6027-6037. doi:doi:10.1128/AEM.01508-09

Sutherland, I. W. (1999). Biofilm Exopolysaccharides. In J. Wingender, T. R. Neu, and H.-C. Flemming (Eds.), *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function* (pp. 73-92). Berlin, Heidelberg: Springer Berlin Heidelberg.

Vance, T. D. (2019). *Adhesion Proteins: Keeping Bacteria in Their Place.* Queen's University (Canada).

Zhang, Z., Schwartz, S., Wagner, L. and **Miller, W.** (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational biology: A Journal of Computational Molecular Cell Biology,* **7**(1-2): 203-214. doi:10.1089/10665270050081478

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J. and **Hwang, G.** (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology,* **9**. doi:10.3389/fbioe.2021.643722

Zobell, C. E. and **Allen, E. C.** (1935). The Significance of Marine Bacteria in the Fouling of Submerged Surfaces. *Journal of Bacteriology,* **29**(3): 239-251. doi:10.1128/jb.29.3.239-251.1935

عزل والتعرف على بكتيريا الأغشية الحيوية من تجمع الحشف الدقيق المطوَّر على مواد اصطناعية مغمورة في البحر الأحمر

إدريس عبدالرحمن[1]*، وممدوح طه جمال[1]، وماجد الشاعري[2]، وصالح محمد المعقر[2،3]، وساتيش سانتوسون[1]

[1]قسم الأحياء البحرية، كلية علوم البحار، جامعة الملك عبد العزيز، ص.ب 80207 جدة 21589، و[2]قسم علوم الأحياء، كلية العلوم، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية، و[3]قسم الأحياء، كلية التربية، جامعة البيضاء، البيضاء، اليمن، المملكة العربية السعودية

* iabdulrahman@kau.edu.sa

المستخلص. تعتبر بكتيريا الأغشية الحيوية الرقيقة مستعمرات سطحية أولية تقوم بتجميع الحشف الحيوي البحري على الأسطح المغمورة، وتعتبر في المرحلة المبكرة من الحشف الدقيق. وحيث الحاجة المكتسبة في تصميم العلاج المضاد للحشف، نظرًا لقدرتها على بدء الحشف الحيوي، ودعم الاستعمار اللاحق للحشف الكبير. في هذه الدراسة، تم عزل العديد من بكتيريا الأغشية الحيوية من طبق بتري وأنابيب البولي فينيل كلوريد (PVC) المغمورة في ساحل البحر الأحمر لمدة أسبوع، كما تم عزل البكتيريا المكونة للغشاء الحيوي عن طريقة لوحة الانتشار تحت ظروف قياسية، وتحديدها بواسطة طريقة تسلسل الرئا الريباسي 516. وتم تقييم كل من العزلات من حيث تكوين الأغشية الحيوية نوعيًا باستخدام مقايسة الأنبوب، ثم استخدام فحص لوحة Microtiter لتحديد البيوفيلم الذي تتجه الكائنات الحية المختارة، وتمكن 11 نوعًا من البكتيريا المعزولة من أصل ٢١ من تكوين غشاء حيوي تحت ظروف المختبر. ومعظم العزلات (7 من 11) من جنس *Pseudoalteromonas* وعزلة واحدة من *Halomonas*، و*Marinomonas*، و*Vibrio*، و*Psychrobacter*. وأوضحت الدراسة أن المجتمع البكتيري المكون للأغشية الحيوية على ركائز صلبة في البحر الأحمر متنوع وقادر على تكوين أغشية حيوية على الأسطح في الظروف المعملية. ويمكن استخدام هذه العزلات ككائنات دقيقة مستهدفة لفحوصات إيجاد مضادات للحشف.

الكلمات المفتاحية: بيوفيلم، بكتيريا، الحشف الحيوي، البحر الأحمر.