Acta Bot. Croat. 80 (1), 112–116, 2021
DOI: 10.37427/botcro-2020-031

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Short communication

Phototrophic biofilms and microbial mats from the marine littoral of the central Mediterranean

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Abstract – Phototrophic biofilm and microbial mat communities grow along the rocky coastline of the Maltese islands. This research involved studying phototrophs from the mediolittoral and supralittoral zones over a two-year period and seasonal changes were observed. Attachment of pioneer microorganisms to the porous eroded limestone bedrock was facilitated via a gelatinous matrix composed of exopolymeric substances (EPS). In submerged areas, such as undisturbed rock pools, these progressively formed green or brown compact biofilms, some of which thickened over the spring to form microbial mats via the production of more extensive EPS layers. Microbial mats gradually attained a lighter colouration due to the presence of ultraviolet (UV) screening pigments. In full summer, they were observed to shrink, detach from the exposed substrate, harden and progressively calcify. Biofilm microorganisms survived the harsh summer months in sheltered areas. The major biofilm formers were filamentous non-heterocytous cyanobacteria belonging to the Leptolyngbyaceae, Pseudanabaenaceae and Oscillatoriaceae. Their sheaths were thick, lamellated and often confluent. A higher biodiversity of phototrophs was observed in late autumn and winter, when tufts of heterocytous *Calothrix sp.* grew on thin compact biofilms of *Nodosilinea* sp., *Toxifilum* sp. and *Phormidesmis* spp., while *Lyngbya* spp. trichomes were surrounded by thick brown sheaths. Germlings of green and brown macroalgal species belonging to *Ulva*, *Cladophora* and *Sphacelaria* were embedded in biofilms and microbial mats and gradually grew to form extensive macroalgal covers submerged in rock pools. *Erythrotrichia* sp. filaments colonised the mediolittoral zone and were confined to areas that were exposed to wave action and submerged intermittently. Over the summer, macroalgal coverage diminished and microalgal biofilms and microbial mats prevailed in rock pools.

Keywords: biofilm, cyanobacteria, marine littoral, microalgae, microbial mat, Mediterranean

Introduction

Phototrophic biofilms and microbial mats grow on coastal rocky shores around the Maltese islands. They constitute under-explored communities as there is an overall lack of research about the biotic assemblages of Maltese rocky shores (Schembri et al. 2005) and of the Mediterranean area in general, as evidenced bythe lack of scientific literature published on the subject.

A biofilm can be defined as a self-sustaining community of microorganisms associated with a substrate that is several millimetres thick (Dang and Lovell 2015). On the other hand, microbial mats (also referred to as biomats) are stratified microbial communities, commonly associated with aquatic habitats, which for the purpose of this study were over one centimetre in thickness. Many of the microbial associations within biofilms and microbial mats are symbiotic, which confers a selective advantage on the community as a whole. In phototrophic communities, a significant proportion of the microorganisms are photosynthetic and highly dependent on the presence of light. Research interest in these communities is related to the discovery of new taxa that produce novel enzymes with high biotechnological potential in green environmental solutions and biomedical applications (Prieto-Barajas et al. 2018).

This study consists of a structural exploration of phototrophic biofilms and microbial mats occurring on the rocky shores of the Maltese islands and a morphological descrip-

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tion of the cyanobacterial and algal biodiversity comprising these communities. The main objectives were to identify any seasonal variations and to attempt to further knowledge about the strategies adopted by these communities that enable them to form on hostile surfaces and thus persist throughout different seasons.

Areas of the Maltese coastline colonised by phototrophic communities were visually observed and documented over a two-year period, and representative biofilms and microbial mats were then seasonally sampled and directly observed by light and electron microscopy. To our knowledge, this is the first such study of marine phototrophic biofilms and microbial mats growing on rocky shores in the central Mediterranean region.

Materials and methods

Phototrophic microbial communities growing in the mediolittoral and supralittoral zones of different coastal locations around the Maltese islands were studied over a two-year period starting in autumn 2017 and seasonal changes were visually observed and documented.

Representative biofilms and microbial mats were then sampled from the rocky shoreline around St Julian's Tower in Sliema (35°55'05.6" N, 14°29'57.8" E) and from the coastline of the village of Baħar iċ-Ċagħaq (35°55'06.4" N, 14°29'58.0" E) in Malta during autumn 2017, spring and autumn 2018 and again during spring 2019. Sampling was carried out during autumn and spring, as these were the seasons in which visible changes in biofilm and biomat structure and extent of growth were recorded. Sampling took place during November 2017, May and December 2018 and again during April 2019. Since the growth of endoliths was not expected, sampling of the phototrophic communities was carried out using techniques that were non-invasive to the underlying rock.

The biofilms and microbial mats were transferred to enriched sea water (Provasoli 1968) in Petri dishes and incubated at 20 °C with a photoperiod of 10 hours.

The sampled phototrophic communities and the same phototrophic communities growing in culture were observed by means of light microscopy using an Olympus BX 51 microscope equipped with an Olympus DP-71 digital camera. For transmission electron microscopy (TEM), biofilm microsamples were fixed in 2.5% glutaraldehyde, postfixed in a 1% osmium tetroxide solution, dehydrated in a graded ethanol series, and embedded in epoxy resin (Epoxy 812 Resin Kit, Multilab Supplies, Newcastle upon Tyne, UK). Thin sections were collected on copper grids, stained with uranyl acetate and lead citrate (Reynolds 1963) and were observed using an H-7100 TEM (Hitachi, Tokyo, Japan) operating at 100 kV.

Identification of phototrophic biofilm and biomat-forming organisms was carried out using morphological keys for Cyanobacteria (Komárek and Anagnostidis 1999, 2005, Komárek 2013), Chlorophyta (Ettl and Gärtner 2013, Cormaci et al. 2014, Lange-Bertalot et al. 2017, Škaloud et al. 2018), Phaeophyceae (Cormaci et al. 2012) and Rhodophyta (Cormaci et al. 2017).

Results and discussion

Seasonal changes in biofilm and biomat structure

Different phototrophic communities grew as biofilms and microbial mats in the marine littoral zone (Fig. 1). In submerged areas, such as undisturbed rock pools or salt pans, microorganisms formed green or brown coloured compact biofilms (Fig. 1a). During spring and summer, similar phototrophic communities prevailed. These were characterised by an abundance of microbial mats located in rock pools found in the mediolittoral and supralittoral zones. Towards the end of spring, the warm weather and restricted wave action led to biofilms becoming restricted to rock pools in the mediolittoral zone.

Seasonality was a crucial factor affecting ecological succession. In fact, some microbial communities thickened over spring to form microbial mats via the production of more extensive EPS layers. These gradually attained a lighter colouration, probably due to the presence of EPS and UV screening pigments such as carotenoids and scytonemins in the upper layers. In full summer, both biofilms and microbial mats survived only close to the shore, submerged in rock pools. On the other hand, exposed biofilms appeared shrivelled, fragmented and progressively detached from the rock surface (Fig. 1b). The mediolittoral zone became reduced during summer due to high average temperatures above 28 °C, a UV index above 9 and low average wind speeds of 2.9 km/h. This led to biofilms being restricted to the area of the mediolittoral zone closest to the shore. Biofilm and microbial mat organisms survived only in rock crevices and shaded areas (Fig. 1c). Dry biomats hardened and became progressively calcified. During autumn, succession occurred, when the rock surfaces previously conditioned by biofilm growth, became colonised by macroalgae.

Composition of the phototrophic communities

Microscopic observations showed that these phototrophic biofilms and biomats were highly diverse communities composed of both phototrophic and heterotrophic microorganisms. Each phototrophic community had a distinctive morphology and species composition which depended on the respective microhabitat.

The predominant microorganisms in communities that were submerged from autumn to spring were filamentous cyanobacteria including fine Leptolyngbyaceae filaments belonging to *Leptolyngbya* sp., *Nodosilinea* sp., Pseudanabaenaceae of *Toxifilum* sp. and non-heterocytous *Oscillatoria* spp., *Phormidium* spp. and *Lyngbya* spp. strains. Dense networks of these filamentous cyanobacteria provided structural strength to the biofilms and microbial mats due to their ability to grow intertwined in sheets or bundles of filaments (Fig. 2), in which other organisms, such

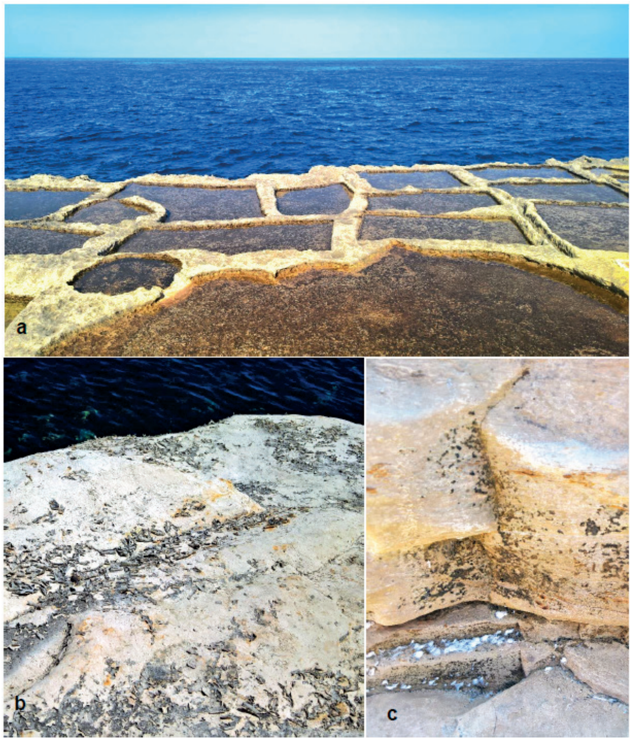

Fig. 1. Dark biofilms grow submerged in man-made salt pans or natural rock pools along the Maltese coastline (a), dry biofilms shrink and completely detach from dry horizontal rock surfaces in full summer (b), biofilm microorganisms survive in crevices and shaded areas on vertical rock faces (c).

as the filamentous heterocytous cyanobacteria *Nunduva* sp. and *Calothrix* sp. (Fig. 2b) and diverse coccal microalgae became embedded. Coccal cyanobacteria included species of *Aphanocapsa* sp. and *Chroococcus* sp., while microalgal strains belonged to species of *Chlamydomonas*, *Chlorella*, *Coelastrella* and *Navicula*. A higher biodiversity of phototrophs was observed in autumn and winter, mainly due to the occurrence of macroalgal germlings in addition to the microbial taxa. The included the filamentous macroalgae *Ulva croatica*, *Ulva flexuosa, Cladophora ruchingeri* and *Sphacelaria* sp. Filaments of *Erythrotrichia* sp. colonised the mediolittoral zone and were mostly confined to areas that were exposed to wave action and submerged intermittently in autumn and winter. Streptomycetes, micronematodes, ciliates and microcrustaceans were also observed living and feeding within the biofilm and biomat communities.

Adaptation and survival strategies

These biofilms and microbial mats formed in response to the prevailing stressful environmental conditions related to temperature, solar irradiation, dehydration and salinity prevalent in the Maltese islands. The layered 3-D structure gave better overall protection than that of constituent microorganisms, such as for instance, the UV protection conferred individually by the cyanobacterium *Lyngbya* and the thermotolerant microalga *Coelastrella*. In such microorgan-

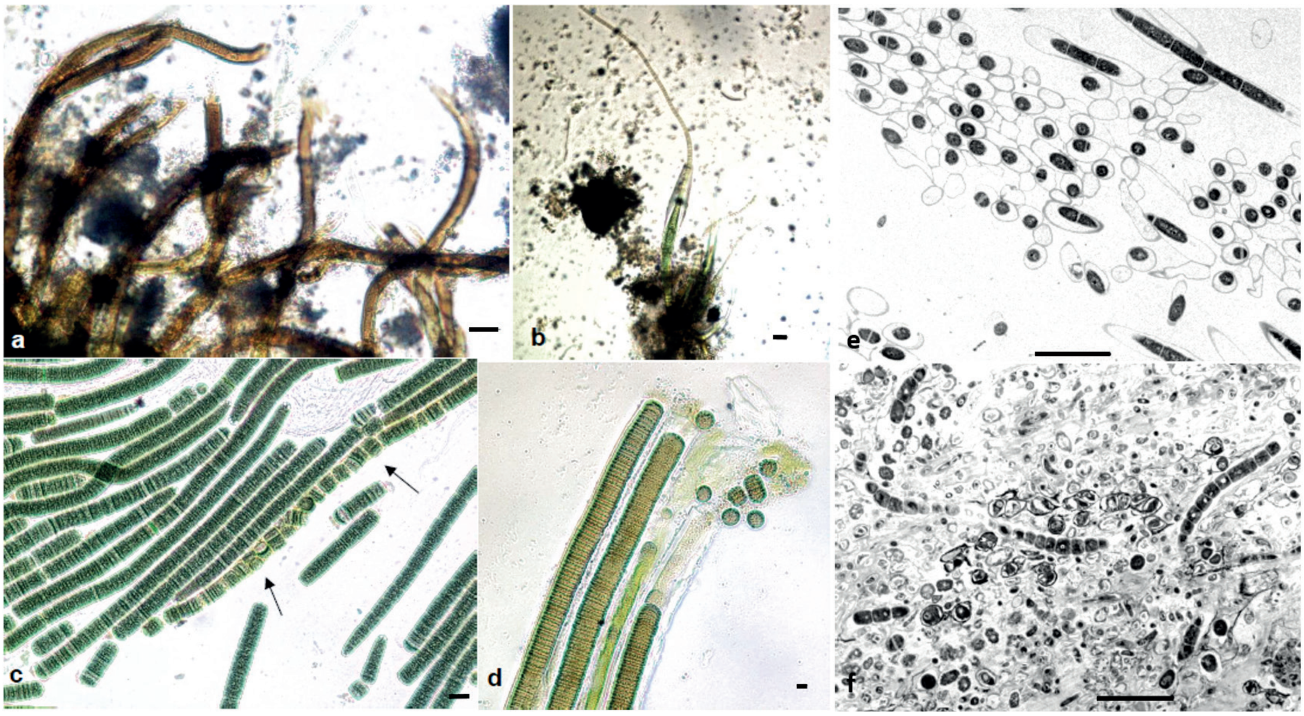

Fig. 2. Biofilm and biomat - forming cyanobacteria *Lyngbya* sp. trichomes surrounded by dark brown sheaths (a), *Calothrix* sp. filaments with hyaline hair (b), arrows indicate profuse trichome fragmentation and formation of hormogonia (c), release of spherical propagules from the open ends of sheaths (d). TEM micrographs of biofilm-forming Pseudanabaenaceae and Leptolyngbyaceae. A biofilm consisting exclusively of fine filaments of *Toxifilum* sp., arranged both longitudinally and horizontally, each surrounded by thick polysaccharide sheaths (e). A different biofilm composed of a more diverse community of *Nodosilinea* sp. filaments with heterotrophic bacteria embedded in a dense EPS matrix (f). Scale bars: 25 µm (a, b), 20 µm (c), 10 µm (d), 5 µm (e, f).

isms, salt and light stress also accelerate carotenoid pigment and oil production (Hu et al. 2013).

Lyngbya aestuarii was common in microbial mat structure. Trichomes in the upper layers of microbial mats were surrounded by a thick brown lamellated sheath (Fig. 2a), which probably contributed to protection against UV radiation (280-400 nm). The filaments formed several separation discs and frequently fragmented into short filaments, which have the potential to grow under favourable conditions (Rath and Adhikary 2007). The species is known to grow within microbial mats present in the marine intertidal zone, in which it performs roles vital to the community and connected to photosynthesis, protection, and hydrogen production, which is considered a key metabolite (Kothari et al. 2013).

Tufts of heterocytous *Calothrix* and *Nunduva* spp. filaments provided for nitrogen (N) fixation. *Calothrix* filaments were heteropolar, with a heterocyte at the basal part of the filament that provided for N fixation and the end of the trichome narrowed into a long hyaline hair to facilitate the uptake of phosphorus (P) (Fig. 2b). Each of these survival strategies provided a competitive advantage which contributed to the success of the biofilm or microbial mat community as a whole.

TEM revealed that the attachment of pioneer microorganisms to the porous eroded limestone bedrock was facilitated via a gelatinous polymeric matrix, in which the whole community ultimately became embedded (Fig. 2e, f). Individual trichomes and cells were surrounded by thick gelatinous sheaths that were often confluent (Fig. 2) and that enabled cells and trichomes to glide in a protected microenvironment that retained the moisture around them (Fig 2f). The production and subsequent release of propagules were frequent (Fig. 2c, d).

The microorganisms were also observed to alter their morphology as a survival strategy to adapt to fluctuating seasonal environmental conditions. One such change was fragmentation observed in *Lyngbya* sp., in which filaments became fragmented into several smaller pieces allowing faster adaptation. Other filaments, especially those belonging to the Leptolyngbyaceae and Oscillatoriaceae were observed to coil and compress, allowing the organism to self-shade within biofilm or biomat structure (Wu et al. 2005).

Microbial mats provided an adequate survival strategy capable of withstanding the harsh environmental conditions prevailing towards the end of spring and summer. In fact, the top protective EPS layer became thicker, allowing microorganisms living in the lower layers to survive. The biomats formed to counteract water shortage, since the larger volume of EPS retains more water via hydrogen bonding, facilitating water absorption and preventing its quick loss, thus allowing the communities to survive in the supralittoral zone, where they are only hydrated by sea spray. The EPS also acted as a buffer preventing rapid micro-environmental changes from occurring within the microbial mat.

Lyngbya aestuarii was often found in the top layers of microbial mats, where it provided UV protection due to its thick lamellated pigmented sheaths that are known to contain scytonemin (Rath and Adhikary 2007). Other microorganisms which required UV protection, such as Leptolyngbyaceae and Rivulariaceae filaments, were found in the bottom layers closer to the substrate.

In fact, another survival strategy involved the migration of filaments, where microorganisms such as Leptolyngbyaceae were able to glide and migrate to the bottom layer of the microbial mat, especially during spring and summer. This strategy has also been recorded and studied in other genera, for instance, *Synechococcus* isolates from Octopus Spring, Yellowstone National Park were observed to migrate away from a strong light source via cell gliding (Ramsing et al. 1997).

The biofilms and microbial mats of the marine littoral constitute unexplored microbial communities in the central Mediterranean region. In fact, they often contain understudied taxa such as those belonging to the genera *Toxifilum* sp. and *Nunduva* sp. that were identified also in this study and have only been recently described (Zimba et al. 2017, González-Resendiz et al. 2018). These microorganisms are currently being investigated via a polyphasic approach, involving the application of molecular genetics and bioinformatics tools to identify relevant species. An improved understanding of the biodiversity and survival adaptations of such phototrophic biofilms and biomats would contribute greatly to our understanding of the role of microbial communities within the coastal ecosystem as a whole.

Acknowledgements

This research was supported by the University of Malta and the ENDEAVOUR Scholarship Scheme (Malta), which is part-financed by the EU's European Social Fund (ESF) under Operational Programme II – Cohesion Policy 2014-2020, Investing in human capital to create more opportunities and promote the wellbeing of society.

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