Società dei Naturalisti in Napoli

Working group on biofilms

Chair:
Emanuela Roscetto

TABLE OF CONTENTS

ISBN 978-88-944878-0-0

Società dei Naturalisti in Napoli

***W**orking **g**roup on **b**iofilms*

International WGB (SNN) Conference:

Microbial growth and novel contrast strategies

Società dei Naturalisti in Napoli
6 July 2020

Società dei Naturalisti in Napoli

Working group on biofilms

SCIENTIFIC COMMITTEES

Antonino Pollio (Università degli Studi di Napoli Federico II, Italy)

Luigi Frunzo (Università degli Studi di Napoli Federico II, Italy)

Marco Guida (Università degli Studi di Napoli Federico II, Italy)

Maria Rosaria Catania (Università degli Studi di Napoli Federico II, Italy)

Emanuela Roscetto (Università degli Studi di Napoli Federico II, Italy)

Antonino De Natale (Università degli Studi di Napoli Federico II, Italy)

LOCAL ORGANIZING COMMITTEES

Emanuela Roscetto (Università degli Studi di Napoli Federico II, Italy)

Book design: Antonino De Natale

Cover pictures: ***A*CUF** (**A**lgal **C**ollection at **U**niversity **F**ederico II)

Preface

On behalf of the Società dei Naturalisti in Napoli and the working group on biofilm, I have the pleasure to welcome you at the workshop entitled "microbial growth and novel contrast strategies", held on July 6, 2020.

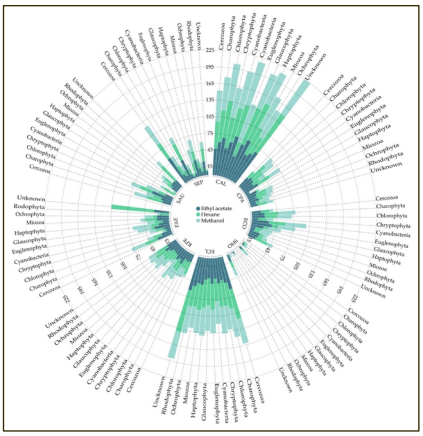
The format of this meeting is based on the idea that workshops based on a limited number of presentations coupled to a webinair system of participation, represent a very efficient and time-saving way of disseminating results and improving discussion. Web-based videoconference is becoming very popular, also due the Covid emergency. They are inexpensive and ensure a high participant satisfaction but cannot be considered as a conference substitute: the average length of online seminars should not exceed 90 minutes, to avoid attention failures. Our intention is to provide a very simple and short format that could stimulate a fruitful collaboration among researchers of this field.

Finally, i want thank the authors of workshop presentation, dr. Emanuela Roscetto, chair of this workshop, that made possible to have this meeting with her excellent scientific and organisational work, and dr. Antonino De Natale for coordinating the production of the proceedings, that will be included on the web site of the Società dei Naturalisti. All future workshop proceedings will be made available on our site and will be indexed.

The workshop organizers and I hope that all participants will enjoy the workshop program.

Società dei Naturalisti in Napoli
President

Società dei Naturalisti in Napoli
6 July 2020



ANTIBIOFILM MOLECULES FROM MICROALGAE

Sara M. Soto[1,*]

[1] Barcelona Institute for Global Health ISGlobal), Barcelona, Spain.

* Sara.soto@isglobal.org

Introduction

Bacterial biofilms are complex biological systems that are difficult to eradicate at a medical, industrial, or environmental level. Biofilms confer bacteria protection against external factors and antimicrobial treatments. Taking into account that about 80% of human infections are caused by bacterial biofilms, the eradication of these structures is a great priority. Biofilms are resistant to old-generation antibiotics, which has led to the search for new antimicrobials from different sources, including deep oceans/seas. The Nomorfilm project (Grant Agreement 634588) was directed to search for new antibiofilm biomolecules from microalgae to apply them to medical devices.

Materials and methods

The initial screening was performed by broth micro-dilution assay to test a single high concentration against all test strains.

Subsequently we determined Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of selected compounds and their ability to prevent biofilm formation using crystal violet staining. The interactions between the selected metabolites were also evaluated by the checkerboard method. Cytotoxicity tests were performed on HaCaT cells.

Results and Discussion

675 extracts obtained from 225 cyanobacteria and microalgae species (11 phyla and 6 samples belonging to unknown group) were obtained from different culture collections: The Blue Biotechnology and Ecotoxicology Culture Collection (LEGE-CC), the Coimbra Collection of Algae (ACOI) from Portugal, and the Roscoff Culture Collection (RCC) from France. Extracts were obtained using a three-solvent protocol that includes hexane, ethyl acetate and methanol.

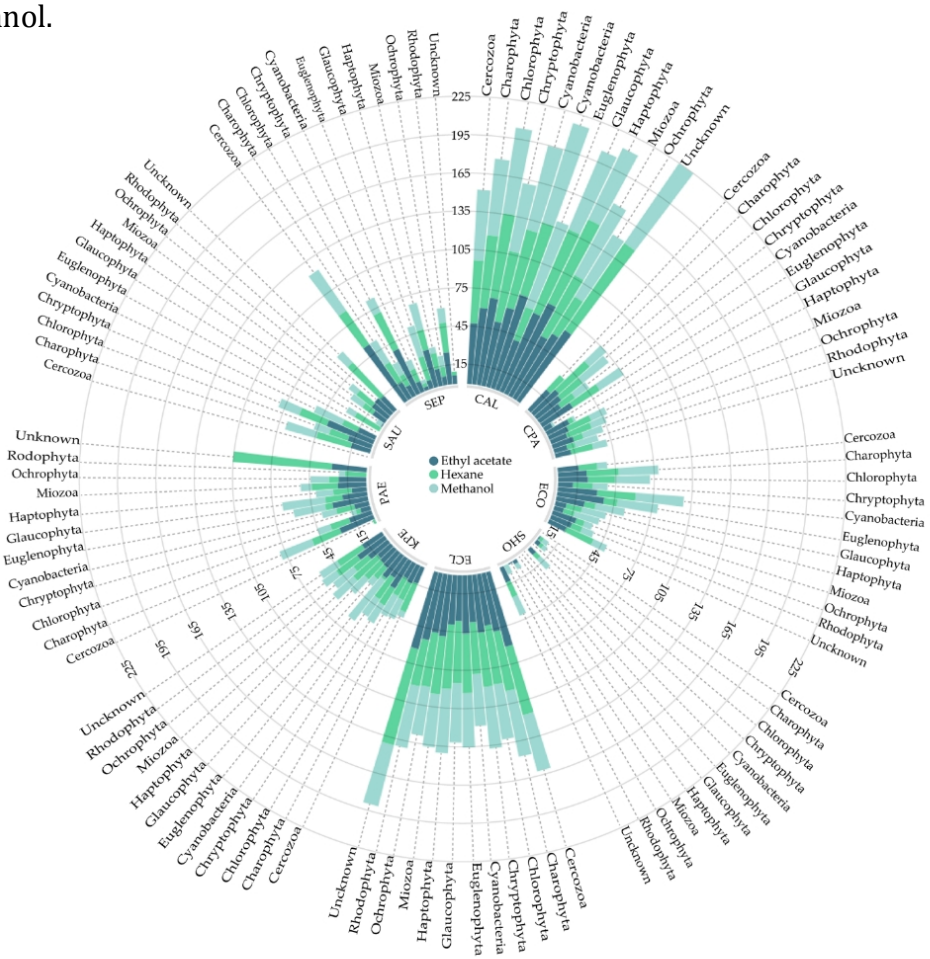


Figure 1. Circular dot plot representing the biofilm inhibition ratio (%) of each bacterium in relation to the solvent employed (ethyl acetate, hexane, and methanol), according to the microalgae and cyanobacteria phylum. CAL: *C. albicans*; CPA: *C. parapsilopsis*; ECO: *E. coli*; SHO: *S. hominis*; ECL: *E. cloacae*; KPE: *K. pneumoniae*; PAE: *P. aeruginosa*; SAU: *S. aureus*; SEP: *S. epidermidis*.

The antibiofilm activity of extracts was determined against seven different bacterial species and two Candida strains in terms of minimal biofilm inhibitory concentration (MBIC) by using the crystal violet protocol.

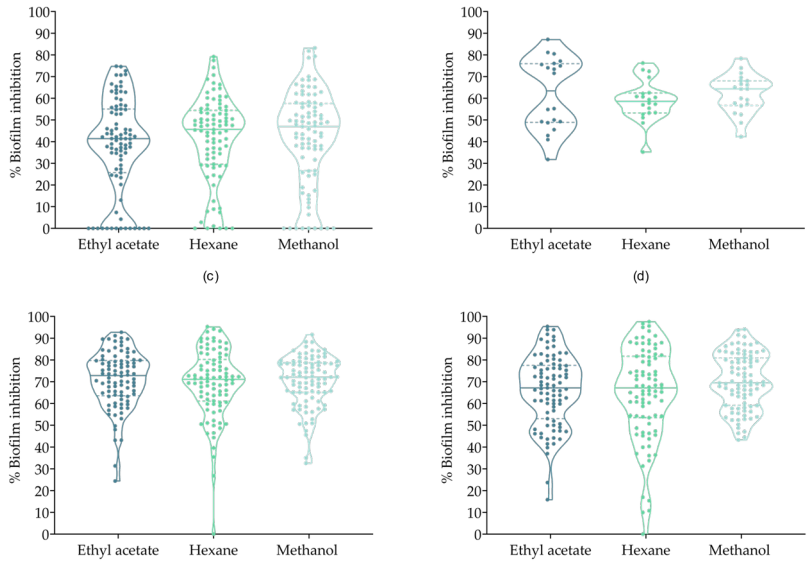


Figure 2. Individual biofilm inhibition ratios of *Charophyta*, *Chlorophyta,* and Cyanobacteria extracts against *E. cloacae* and *C. parapsilopsis*, represented as percentages. (**a**): *Charophyta* against *E. cloacae*; (**b**) *Chlorophyta* against *E. cloacae*; (**c**) Cyanobacteria against *E. cloacae*; (**d**) *Charophyta* against *C. albicans*; (**e**) *Chlorophyta* against *C. albicans*; (**f**) *Cyanobacteria* against *C. albicans.*

Conclusions

The present study provides initial large-scale evidence that microalgae and cyanobacteria are rich sources of substances with antibiofilm activity. Large-scale screening programs like this are extremely useful for identifying organisms that warrant further study as producers of bioactive substances of interest. Overall, the findings of this study provide insights for new opportunities provided by oceans and freshwater systems in the fight against biofilm infections. Further studies will be made in order to determine the active compounds responsible of the antibiofilm activity as well as their toxicity to mammal cells because at extract level toxicity could be due to other components different from the active one.

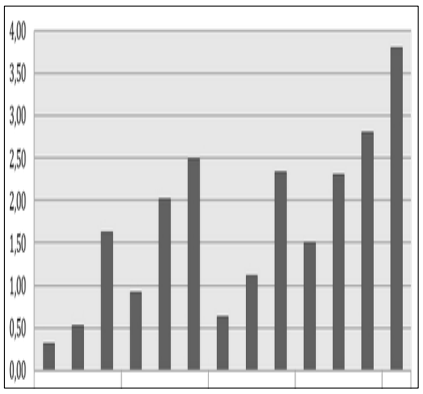
References

Donlan, R.M.; Costerton, J.W. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev., 2002, 15: 167-193.

Blunt, J.W.; Copp, B.R.; Keyzers, R.A.; Munro, M.H.G.; Prinsep, M.R. Marine natural products. Nat. Prod. Rep., 2017, 34: 235-294.

Shannon, E.; Abu-Ghannam, N. Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. Mar. Drugs, 2016, 14(81): 1-23.

De Morais, M.G.; Vaz, B.D.S.; De Morais, E.G.; Costa, J.A.V. Biologically active metabolites synthesized by microalgae. Biomed. Res. Int., 2015, 2015.



USE OF SANITISERS IN THE ERADICATION OF MICROBIAL BIOFILMS ON DIFFERENT MATERIALS

Federica Carraturo[1,2,*], Marco Guida[1,2]

[1] Laboratori di Igiene: Acqua, Alimenti, e Ambiente - Dipartimento di Biologia, Università degli Studi di Napoli Federico II
[2] Laboratorio di Igiene - Centro Servizi Metrologici e Tecnologici Avanzati, Università degli Studi di Napoli Federico II
* federica.carraturo@unina.it

Introduction

Bacteria colonizing toilets tend to remain in the bowl after flushing, forming biofilms. Biofilms cause serious issues, and the augmented resistance of biofilm-associated microorganisms to antimicrobial actives is responsible of serious infections (Bridier et al., 2011). On toilet surfaces, gut-associated taxa were prevalent: human-associated strains, including several taxa associated with the gut, mouth and urine are generally isolated (Flores et al., 2011; Adewoyin et al., 2013) for large periods of time (Barker and Jones, 2005), representing high risk for human health. The purpose of the research was to assess the capability of 4 commercially available detergency products of eradicating multi-strains microbial biofilms from surfaces of plastic and porcelain. The anti-biofilm efficacy was evaluated at different times of action, additionally applying decreasing concentrations of the products under examination. Biofilm eradication capability test and residual viable microorganisms evaluation resulted useful to establish the best product for microbial biofilm treatments.

Materials and methods

E. coli ATCC® 25922, *E. faecalis* ATCC® 19433, *S. Typhimurium* ATCC® 14028, *P. aeruginosa* ATCC® 9046, and *S. aureus* ATCC® 25923 were used to develop a multi-strain biofilm over 96 h. The biofilm on each minimum biofilm eradication concentration sample contained 10^8 UFC/mL of the five bacteria. The detergents evaluated were the following: P1, P2, P3, P4. The anti-microbial actives evaluated were, respectively: sodium hypochlorite and amine oxide (P1), hydrogen peroxide (P2), benzalkonium chloride (P3), oxygen based bleach (P4). Biofilm removal was evaluated using viable count (in compliance with EPA MLB SOP

MB-20 method, “Single Tube Method for Determining the Efficacy of Disinfectants against Bacterial Biofilms”), microbial load at 560 nm, biomass quantitation at 570 nm. Analysis were conducted in triplicate.

Results and Discussion

Biofilm eradication capability test was tested on the following settings:

Decreasing concentrations (100%, 50%, 20%), one time of contact (20 min)

100% concentration, different times of contact (5, 10, 15, 20 min)

100% concentration, 20 min time of contact

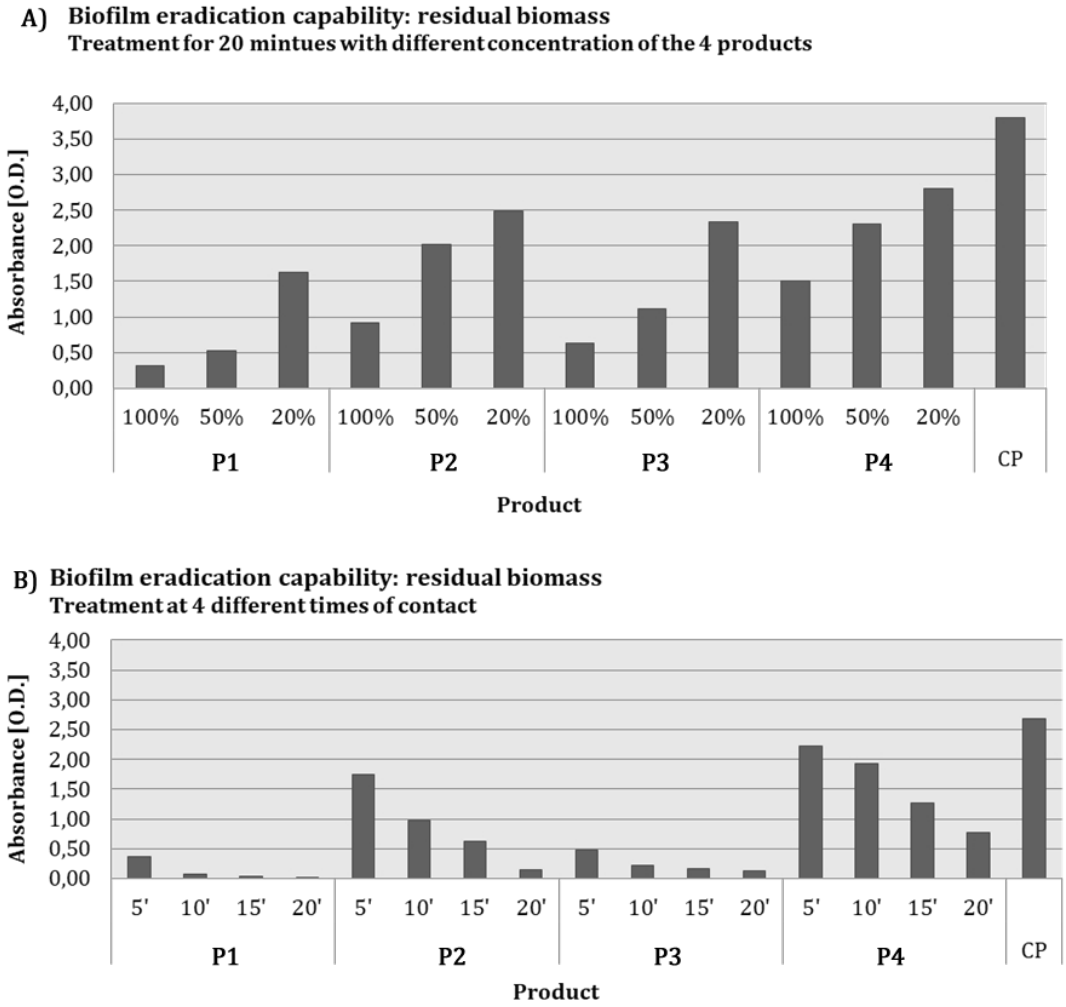


Figure 1. Biofilm eradication capability: residual biomass on plastic. A) Treatment for 10 mintues with different concentrations of the 4 products (P1, P2, P3, P4). B) Products at 100% concentrations for different times of action (5, 10, 15, 20 minutes). CP is the non-treated positive control.

Results allow to hypothesize that chlorine based detergents show higher performances in reducing the viable bacterial load and removing the bacterial EPS, compared to oxygen peroxide based products. P1 showed the highest bactericidal and biofilm eradication activity, both at decreasing concentrations and different times of contact (Figure 1). On plastic matrix, P1 eradicates microbial biofilms respectively up to 20x more than the other formulates (Figure 2); on porcelain P1 eradicates microbial biofilms 10x more than P2, P3, and P4 (Figure 3).

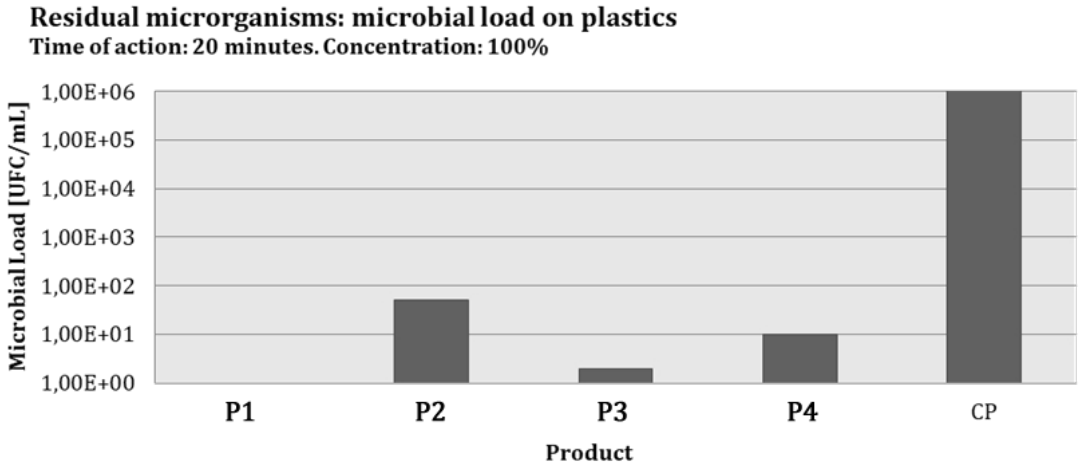


Figure 2. Biofilm eradication capability: residual viable microorganisms on plastic. Treatment employing products (P1, P2, P3, P4) at 100% concentrations under 20 minutes application. CP: non-treated positive control.

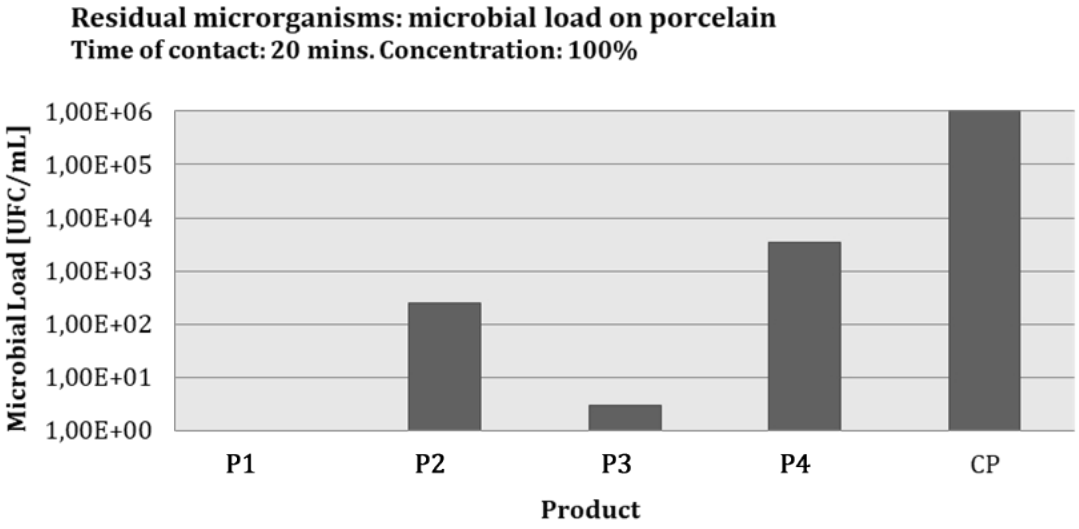


Figure 3. Biofilm eradication capability: residual viable microorganisms on porcelain coupons. Treatment employing products (P1, P2, P3, P4) at 100% concentrations under 20 minutes application. CP: non-treated positive control.

Conclusions

P1 anti-biofilm and antibacterial activities on plastic and porcelain matrices tended to be higher than those of P2, P3, and P4. Outcomes therefore suggest that P1 is the most powerful disinfectant among the four tested.

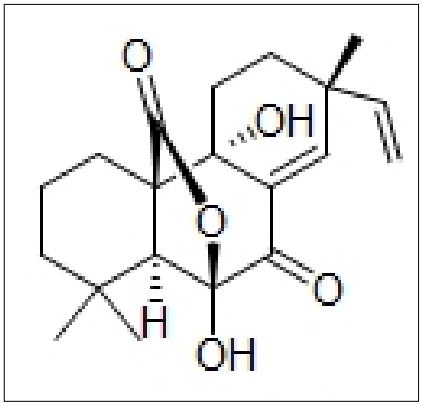
References

Adewoyin, A.G.; Majolagbe, O.N.; Ogunmoyewa, T.; Abejide, M.A. Antibiotic Resistance Profile of Microbial Isolates of Toilet-Bowl of Some Students' Hostels in Ogbomoso, Nigeria. Eur. J. Appl. Sci., 2013, 5 (3): 76-79.

Barker, J.; Jones, M.V. The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. J. Appl. Microbiol., 2005, 99: 339-347.

Bridier, A.; Briandet, R.; Thomas, V.; Dubois-Brissonnet F. Resistance of bacterial biofilms to disinfectants: a review. Biofouling, 2011, 27: 1017-1032.

Flores, G.E.; Bates, S.T.; Knights, D.; Lauber, C.L.; Stombaugh, J.; Knight, R.; Fierer, N. Microbial Biogeography of Public Restroom Surfaces. PLoS One, 2011, 6(11): e28132.



ANTIBACTERIAL SECONDARY METABOLITES FROM PATHOGENIC FUNGI OF FOREST TREES. ANTI-BIOFILM ACTIVITY OF THE PIMARANE DITERPENE SPHAEROPSIDIN A AGAINST CLINICAL ISOLATES OF ANTIBIOTIC-RESISTANT BACTERIA

Marco Masi[1,*], Emanuela Roscetto[2,**]

[1] Dipartimento di Scienze Chimiche, Università di Napoli Federico II, Complesso Universitario Monte S. Angelo, Naples, Italy.
[2] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy.
* marco.masi@unina.it
** emanuela.roscetto@unina.it

Introduction

Many pathogens involved in human infection rapidly increased their antibiotic resistance reducing the effectiveness of therapies in recent decades. Most of them can form biofilms and effective drugs are unavailable to treat these formations. Natural products could represent an efficient solution to overcome the antimicrobial resistance and treat biofilm-related infections (Roscetto et al., 2018; Vila et al., 2019). In this study, 20 secondary metabolites produced by pathogenic fungi of forest plants (Masi et al., 2018) were evaluated for the first time against antibiotic-resistant staphylococci and *P. aeruginosa*.

Materials and methods

The initial screening was performed by broth micro-dilution assay to test a single high concentration against all test strains.

Subsequently we determined Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of selected compounds and their ability to prevent biofilm formation using crystal violet staining. The interactions between the selected metabolites were also evaluated by the checkerboard method. Cytotoxicity tests were performed on HaCaT cells.

Results and Discussion

The cyclohexene oxides epi-epoformin and sphaeropsidone, and the pimarane diterpenoid sphaeropsidin A (Fig. 1) showed activity on all test strains (Tab. 1). Particularly, sphaeropsidin A was effective at low concentrations with MIC values ranging from 6.25 µg/mL to 12.5 µg/mL. Mixtures of sphaeropsidin A and epi-epoformin have shown antimicrobial synergistic effects with a concomitant reduction of cytotoxicity against HaCaT cells.

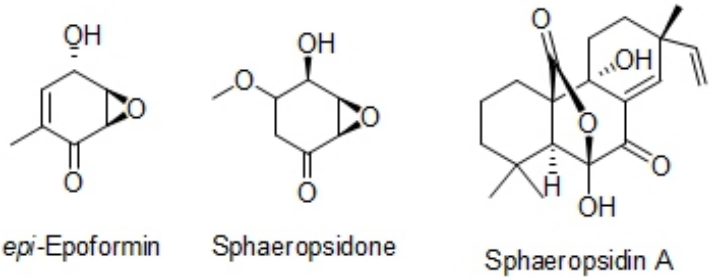


Figure 1. The structures of epi-epoformin, sphaeropsidone, and sphaeropsidin A.

Furthermore, sphaeropsidin A at sub-inhibitory concentrations decreased MRSA and P. aeruginosa biofilm formation. A biofilm-focused therapeutic approach reducing the ability of these pathogens to form biofilms would decrease the antibiotic recalcitrance of these infections, thus allowing treatment with the antibiotics in use and a faster and more effective healing.

Table 1. MIC (µg/mL) and MBC (µg/mL) of epi-epoformin, sphaeropsidone, and sphaeropsidin A against Gram-positive and Gram-negative test strains.

Bacterial strain	*epi*-epoformin		sphaeropsidone		spaheropsidin A		Amikacin		Teicoplanin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 43300	100	100	100	>200	12.5	100	nt	nt	1	4
MRSA 1118-116	100	100	100	>200	6.25	25	nt	nt	0.5	4
S. haemolyticus ATCC 29970	100	100	100	>200	12.5	50	nt	nt	2	>4
S. haemolyticus VR 1219-118	100	100	100	>200	12.5	50	nt	nt	2	>4
P. aeruginosa PAO1	50	>200	>100	>200	12.5	>200	4	32	nt	nt
P. aeruginosa 0418-925	50	>200	100	>200	12.5	>200	16	>32	nt	nt

To our knowledge, this is the first report on the anti-biofilm activity of sphaeropsidin A.

In our opinion the screening performed represents a promising basis for identifying scaffolds with antimicrobial potential. The characterization of the action mechanism of these compounds and the introduction of chemical modifications in their molecules could lead to the synthesis of derivatives with acceptable biocompatibility and improved antimicrobial properties against multi-resistant and biofilm-producing bacteria.

Conclusions

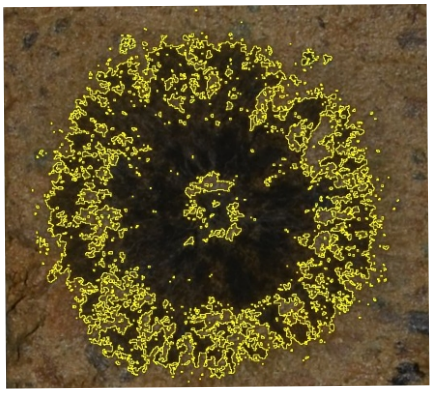
Our results represent preliminary data on the antimicrobial activity of fungal secondary metabolites evaluated for the first time against common opportunistic pathogen inducing human heavy infections. The activity of sphaeropsidin A appears noteworthy for its ability to inhibit biofilm formation. Further experiments will be targeted at the development of biocompatible formulations of epi-epoformin and sphaeropsidin A suitable for wound treatment to prevent the development of serious infections.

References

Masi, M.; Maddau, L.; Linaldeddu, B. T.; Scanu, B.; Evidente, A; Cimmino, A. Bioactive metabolites from pathogenic and endophytic fungi of forest trees. Curr. Med. Chem., 2018, 25: 208–252.

Roscetto, E.; Contursi, P.; Vollaro, A.; Fusco, S.; Notomista, E.; Catania, M.R. Antifungal and anti-biofilm activity of the first cryptic antimicrobial peptide from an archaeal protein against Candida spp. clinical isolates. Sci. Rep., 2018, 8: 17570.

Vila, J.; Moreno-Morales, J.; Ballesté-Delpierre, C. Current landscape in the discovery of novel antibacterial agents. Clin. Microbiol. Infect., 2019, 26(5): 596-603.



FUNGI ON LITHIC SUBSTRATA: IN VITRO ASSESSMENT OF COLONIZATION PATTERNS

Mariagioia Petraretti[1,*], Angelo Del Mondo[1,2], Antonino De Natale[1,3], Antonino Pollio[1,3,**]

[1] Laboratorio di Biologia delle Alghe - Dipartimento di Biologia, Università degli Studi di Napoli Federico II.

[2] Stazione Zoologica Anton Dohrn, Istituto Nazionale di Biologia, Ecologia e Biotecnologie marine, Villa Comunale, 80121 Napoli, Italy.

[3] Metodologie Analitiche per la Salvaguardia dei Beni Culturali (Masbc), Task Force d'Ateneo Federico II di Napoli, Complesso Universitario di Monte Sant'Angelo, Naples, Italy.

* mariagioia.petraretti@unina.it

** anpollio@unina.it

Introduction

Stone biodeterioration is a topic of increasing concern and the study of microbial biofilm contribution to biodeterioration phenomena is crucial to better preserve monuments worldwide (Dakal and Cameotra, 2012). Particularly, fungi play a key role in biodeterioration of monuments, causing both physical and chemical damages of stone surfaces (Cuzman et al., 2011). Campania, Italy, hosts a large number of stone artworks, spanning over three thousand years. Despite this unique cultural heritage, a deep sampling aimed at assessing the biodiversity of fungi and their fine-scale distribution has never been conducted in this region. The study of fungal biodiversity on stone monuments is a preliminary step to better understand biodeterioration phenomena and thus to preserve monuments worldwide. In order to reproduce biodeterioration of fungal strains on lithic substrata, an *in vitro* approach has been assessed, starting from most common isolates of our sampling campaign.

Materials and methods

Sampling

Sampling was focused on some of the most important archeological sites of Campania, namely Oplontis, Hercolanuem and Pompeii, three cities destroyed by the Vesuvius eruption of 79 AD. Sampling points were chosen on the basis of the visibility of alteration in stone surfaces. Samples were taken with a sterile cotton swab and also by applying adhesive tape on the stone surface, then stored at 4° C.

Isolation and Identification

Samples were cultivated using as medium Potato Dextrose Agar. Mycelia were isolated with the aid of a stereomicroscope. Then, fungi were separately cultivated and observed with the optical microscope. The identification of fungi was based on the macroscopic features of colonies and morphology of the reproductive structures coupled with molecular analyses.

In vitro experiments

Sterile tuff tiles were displaced in triplicate in autoclaved glass chambers and then watered with sterile Bold's Basal Medium added with sucrose 12g/L. Spores of most common fungi sampled (*Fusarium oxysporum*, *Aspergillus niger*, *Alternaria alternata*) were obtained from old colony treated for 1 min with Tween20 0,5%. The spore suspension was recovered and diluted and the number of spores per milliliter was determined in a Bürker blood-counting chamber. The spores suspended in sterile distilled water, were injected in the middle of each tile. One more glass chamber was prepared with tuff tiles watered with distilled water instead of nutritive medium and kept until the end of the experiment as a control. Fungal growth was analyzed every 4 days.

Results and Discussion

Metallurgical microscopy measurements for thickness and surface overlay were used to monitor fungal growth on central, median and distal regions of tuff tiles. Although Carrara marble and Pietra di Lecce are the most studied Italian lithotypes (Miller at. al., 2012), yellow tuff is one of the most common building stone of Campania, since Antiquity (Calcaterra et al., 2012).

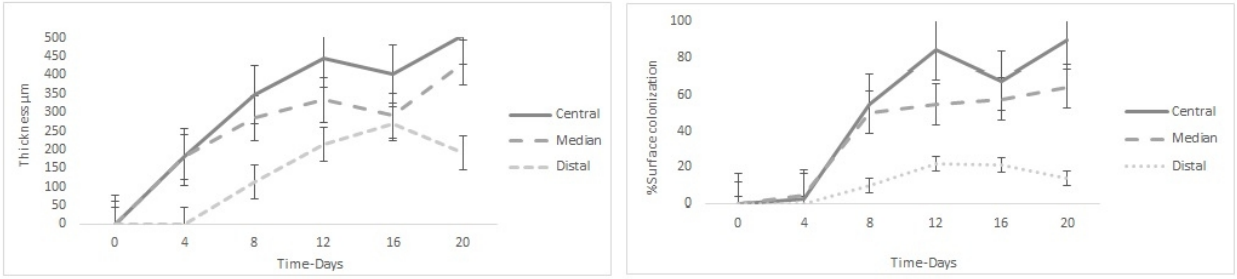


Figure 1. Percentage of thickness of *Alternaria alternata* biofilm growth on tufa tiles (left), surface colonization on tufa tiles (right); points: central (––), median (- - -), distal (· · ·).

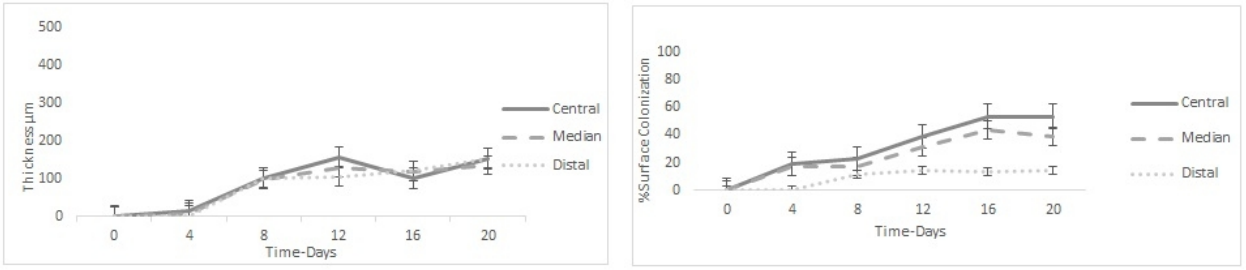


Figure 2. Percentage of thickness of *Fusarium oxysporum* biofilm growth on tufa tiles (left), surface colonization on tufa tiles (right); points: central (––), median (- - -), distal (· · ·).

In *A. alternata* after 16 days incubation both thickness and overlay decrease in central and medial regions, while in distal region this trend appears after 20 days incubation. In *F. oxysporum* the maximum average thickness appears to be fixed to about 200 µm for all the considered regions.

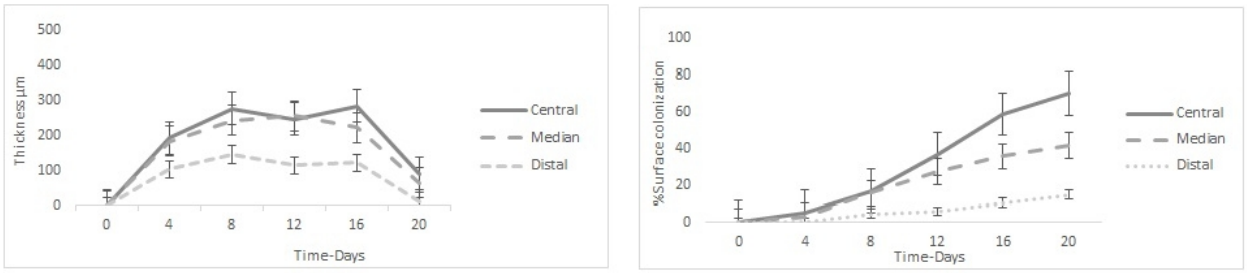


Figure 3. Percentage of thickness of *Aspergillus niger* biofilm growth on tufa tiles (left), surface colonization on tufa tiles (right); points: central (––), median (- - -), distal (· · ·).

A similar pattern was also observed in the median region, albeit shifted at the 20th day, whereas in the distal region growth was lower, and the hyphae were unable to colonize all available space. *A. niger* shows exponential growth for overlay surface, after 12 days incubation thickness decrease in all regions analyzed. We have hypothesized that both parameters may be influenced by limiting available nutrients (Giannantonio, 2009).

Conclusions

In vitro experiments are promising tools for the study of biodeterioration and to better preserve UNESCO World Heritage site monument. Kept in colture a wide range of cultivable fungi isolated directly from studied area, will allow to better identify mode of action of fungi on stones and to develop integrated and adaptive management plans, based on the use of "green" substances.

References

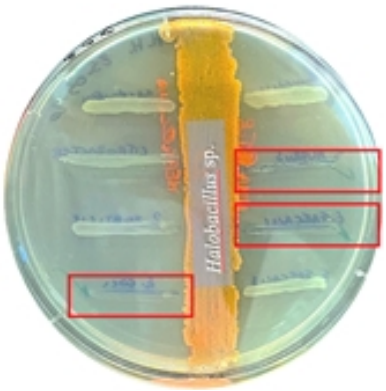
Calcaterra, D.; Cappelletti, P.; Colella, A.; De'Gennaro, M.; De Gennaro, R.; Langella, A. Le pietre delll'architettura storica della Campania. Arkos: Scienza e Restauro dell'Architettura, 2003, 4(2): 40-46.

Cuzman, O.A.; Tiano, P.; Ventura, S.; Frediani, P. Biodiversity on stone artifacts. The importance of biological interactions in the study of biodiversity. 2011 Sep 22: 367-390.

Dakal, T.C.; Cameotra, S.S. Microbially induced deterioration of architectural heritages: routes and mechanisms involved. Environmental Sciences Europe, 2012 Dec 1, 24(1): 36.

Giannantonio, D.J., Kurth, J.C., Kurtis, K.E., Sobecky, P.A. Effects of concrete properties and nutrients on fungal colonization and fouling. International Biodeterioration & Biodegradation. 2009 Apr 1, 63(3): 252-259.

Miller, A.Z., Sanmartín, P., Pereira-Pardo, L., Dionísio, A., Sáiz-Jiménez, C., Macedo, M.F., Prieto, B. Bioreceptivity of building stones: a review. Science of the Total Environment, 2012 Jun 1, 426: 1-2.



ANTIMICROBIAL PRODUCTION CAPACITY OF THE TYRRHENIAN COASTAL MICROBIOTA: GENOMIC INFERENCES

Paulina Corral[1,2,*]

[1] University of Naples Federico II, Dept. of Biology, 80126 Naples - Italy

[2] University of Sevilla, Faculty of Pharmacy, Dept. Microbiology and Parasitology, 41012 Sevilla – Spain

* pcv@us.es

Introduction

The Tyrrhenian sea is characterized by submarine volcanos and noted for its beauty and crystalline waters. Located in western coast of the Mediterranean Sea of Italy, it is one of the most visited places for summer tourism where the human impact and weather conditions alter the microbial composition. We hypothesize that these both conditions play an essential role in the microbiota leading to metabolic changes and stimulating its biosynthetic capacity of novel compounds in a different yield, fact evidenced in winter and summer. The goal of the project is the identification of new antimicrobial molecules from marine microorganisms in soil and water based on multi-omics approach for the sustainable development of lead compounds to face the increasing of the antimicrobial resistance.

Materials and methods

The sampling points for this study are Miseno and Varcaturo. Both places highly crowded in summer that represent a suitable feature for tracking the human influence and climatic conditions on the marine microbiota for the antimicrobial production. Water and seashore sediments were collected in Winter (February 2020). Samples were 10-fold diluted and inoculated in minimum medium specifically designed to mimic environmental conditions. The antimicrobial screenings were performed by well-diffusion method and cross streaking against human pathogens. The closest genomes available genomes of the related closest species available were studied using the tool antiSMASH tool in order to identify the biosynthetic gene clusters (BGCs) linked to the activity observed *in vitro*.

Results and Discussion

From the bioactive strains, 3 isolates were identified based on 16S rRNA gene sequence. The closest similarity with species of the genus *Halobacillus* sp., *Jeotgalibacillus* sp. and *Catenovulum* sp. respectively, all with values lower than 97% and representing new species.

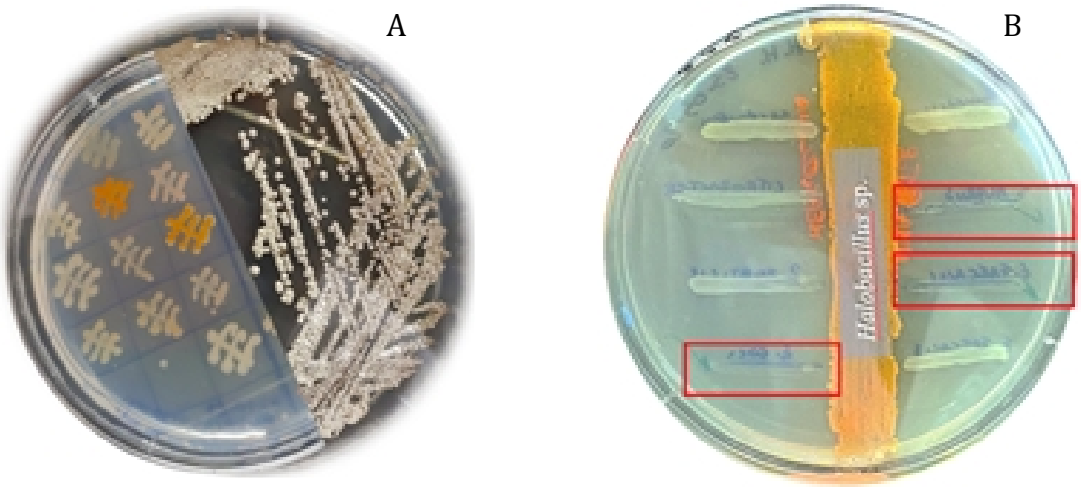


Figure 1. A) Patch and isolation of *Halobacillus sp.* in minimal media. B) Cross-streaking showing the inhibitory effect of *Halobacillus* sp.against *Escherichia coli*, *Staphilococcus aureus*, *Enterococcus faecalis*.

The most active strain is *Halobacillus* sp. strain V26 with inhibitory effect against *Escherichia coli*, *Staphilococcus aureus* and *Enterococcus faecalis*. The genome available of the closest relative: *Halobacillus dabanensis* displays a secondary metabolite biosynthetic gene cluster that match with Betalactone with 46% of similarity with Fengycin that is a Non-Ribosomal Peptide. The results obtained in winter demonstrate that the antimicrobial capacity is related to the novelty of the isolates giving a hint for further studies and discarding already known producers.

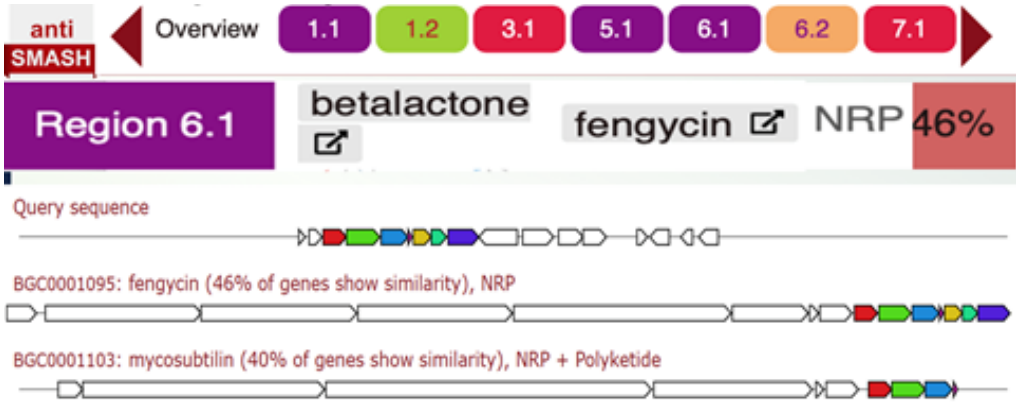


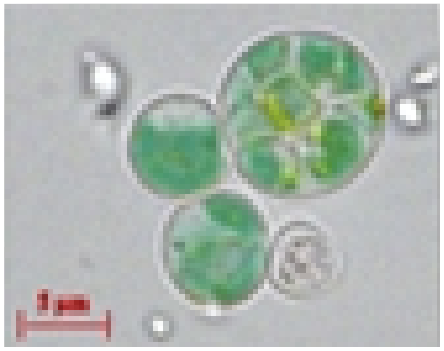
Figure 2. Genome analysis BGC of the closest relative *Halobacillus dabanensis* showing the region 6.1 identified as a betalactone with 46% of similarity with fengycin.

Conclusions

The Tyrrhenian strait, in particular, the cost of Flegraean Fields constitutes a promising resource for novel microorganism with prolific antimicrobial capacity. In summer it is expected that the yield of isolation of novel strains and their bioactivity capacity is higher than winter due to the temperatures and changes in the microbiota due to human impact. The results will be contrasted with seasonal metagenomic studies that will reveal how the microbial population is changed.

References

Haro-Moreno, J.M.; López-Pérez, M.; de la Torre, J.R.; Picazo, A.; Camacho, A.; Rodriguez-Valera, F. Fine metagenomic profile of the Mediterranean stratified and mixed water columns revealed by assembly and recruitment. Microbiome, 2018, 6(1): 128.



THE POLYEXTREMOPHILIC RED ALGA *GALDIERIA SULPHURARIA* (CYANIDIOPHYCEAE, RHODOPHYTA) IN CONTRASTING BACTERIAL CONTAMINATION

Manuela Iovinella[1,*], M. Palmieri[2], Claudia Ciniglia[3]

[1] Department of Biology, University of York, York, UK.

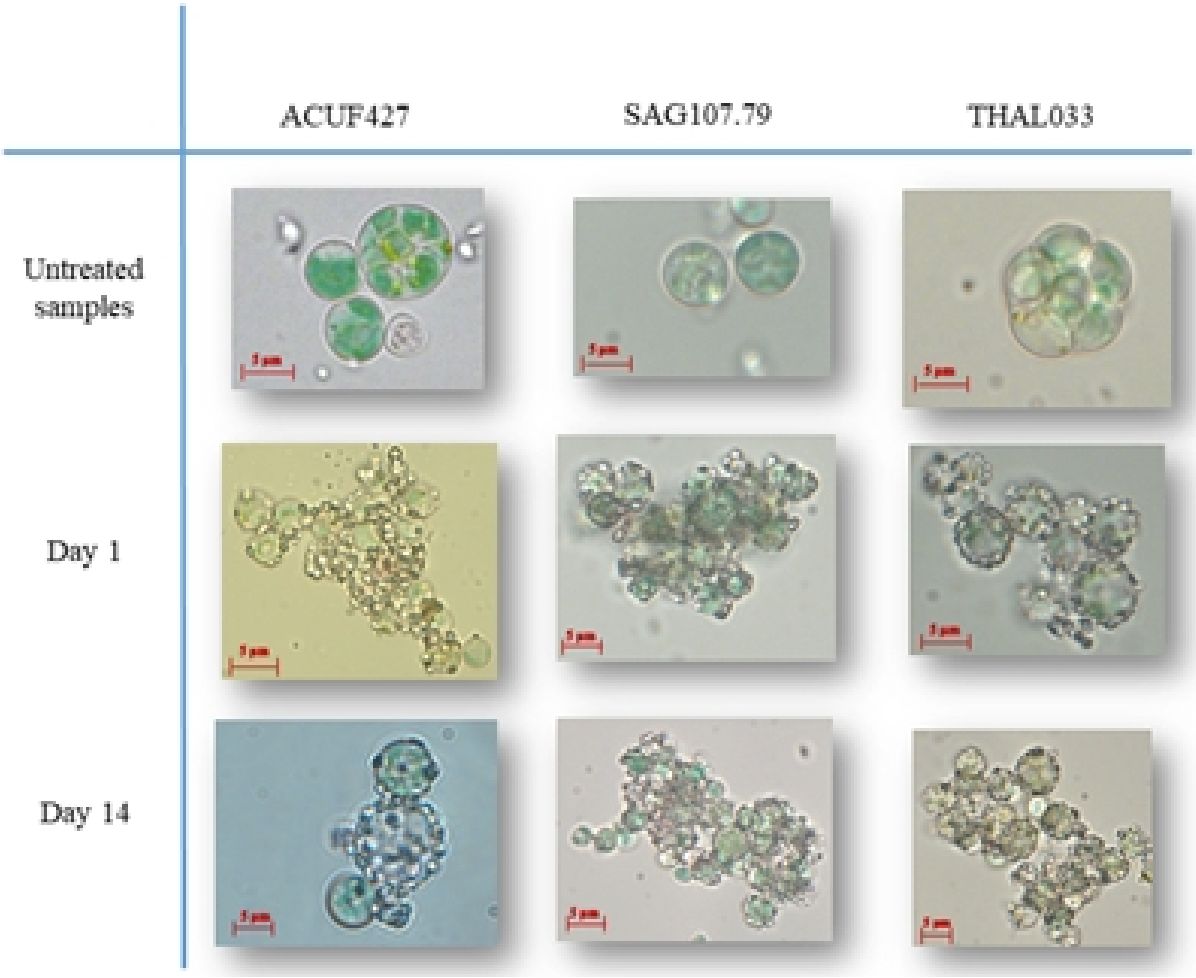
[2] Department of Environmental, Biological and Pharmaceutical Science and Technology, Università della Campania L. Vanvitelli, Caserta, Italy.

* Mi676@york.ac.uk

Galdieria sulphuraria is a thermo-acidophilic, unicellular red alga, dwelling in hot acidic waters, volcanic calderas and anthropogenic acidic environments, such as acidic mine drainage. This alga is able to grow photoautotrophically, mixotrophically, and heterotrophically; it thrives on more than 50 different carbon sources, such as sugars, sugar alcohol and amino acids, thus conferring it a metabolic flexibility comparable to a bunch of few other microorganisms (Rigano et al., 1976; Oesterhelt et al., 1999). This feature, coupled with the resistance to low pH, high temperature, salinity, metal toxicity, and other abiotic stressors motivate the choice of G. sulphuraria for several biotechnological purposes and applications. As other extremophile microalgae, G. sulphuraria is an optimal potential candidate for large-scale production, since the extreme growth conditions help avoiding contamination, especially in open pond reactors (Delanka-Pedige et al., 2019). The present report is a literature review on the capacity to contrast bacterial contamination, reduce pathogen attack and remove antibiotic resistant genes and bacteria by employing G. sulphuraria in algal-based wastewater treatment system. The microbial population decline from 99% to 17% in algal systems, with complete removal of Enterococcus faecalis and Escherichia coli in the algal system. The coliform inactivation observed in algal wastewater treatment systems is related to the synergistic effect of more than one factors, namely pH, temperature, light and algal biomass (Delanka-Pedige et al., 2019). Moreover, compared to conventional treatment systems, in the G. sulphuraria based-system the decrement in concentrations of erythromycin- and sulfamethoxazole-resistant bacteria in the effluent is more effective. A decrease is recorded both in total bacteria and in antibiotic resistant-genes (ARGs), such as qnrA, qnrS, tetW in surviving bacteria, in algal system.

These results support the feasibility of the algal-based wastewater treatment system in decreasing ARGs and ARB and in minimizing the spread of antibiotic resistance to the environment (Munasinghe-Arachchige et al., 2019). Research on the antimicrobial activity could be coupled with the biotechnological assessment of extracellular polymeric substances (EPS). Exposure to stress factors induce the production of exopolysaccharides and exoproteins, whose biotechnological applications concern antitumoral, antiviral and antibacterial activity (Miazek et al., 2015; Xiao et al., 2016). In our laboratory we induced the production of EPS in different strains of *G. sulphuraria*, using the Rare-Earth-Element Cerium (III) as the stressor factor. Ongoing experiments regard the evaluation of the antimicrobial activity of the released EPS (Tab 1).

Table 1. Microscope pictures showing the EPS mantle in samples treate with 125mg/L of Cerium.



References

Delanka-Pedige, H.M.K.; Munasinghe-Arachchige, S.P.; Cornelius, J.; Henkanatte-Gedera, S.M.; Tchinda, D.; Zhang, Y.; Nirmalakhandan, N. Pathogen reduction in an algal-based wastewater treatment system employing Galdieria sulphuraria. Algal Research, 2019, 39: 101423.

Miazek, K.; Iwanek, W.; Remacle, C.; Richel, A.; Goffin, D. Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis: A review. International Journal of Molecular Sciences, 2015, 16 (10): 23929-23969.

Munasinghe-Arachchige, S.P.; Delanka-Pedige, H.M.K.; Henkanatte-Gedera, S.M.; Tchinda, D.; Zhang, Y.; Nirmalakhandan, N.Y. Factors contributing to bacteria inactivation in the Galdieria sulphuraria-based wastewater treatment system, Algal Research, 2019, 38: 101392 .

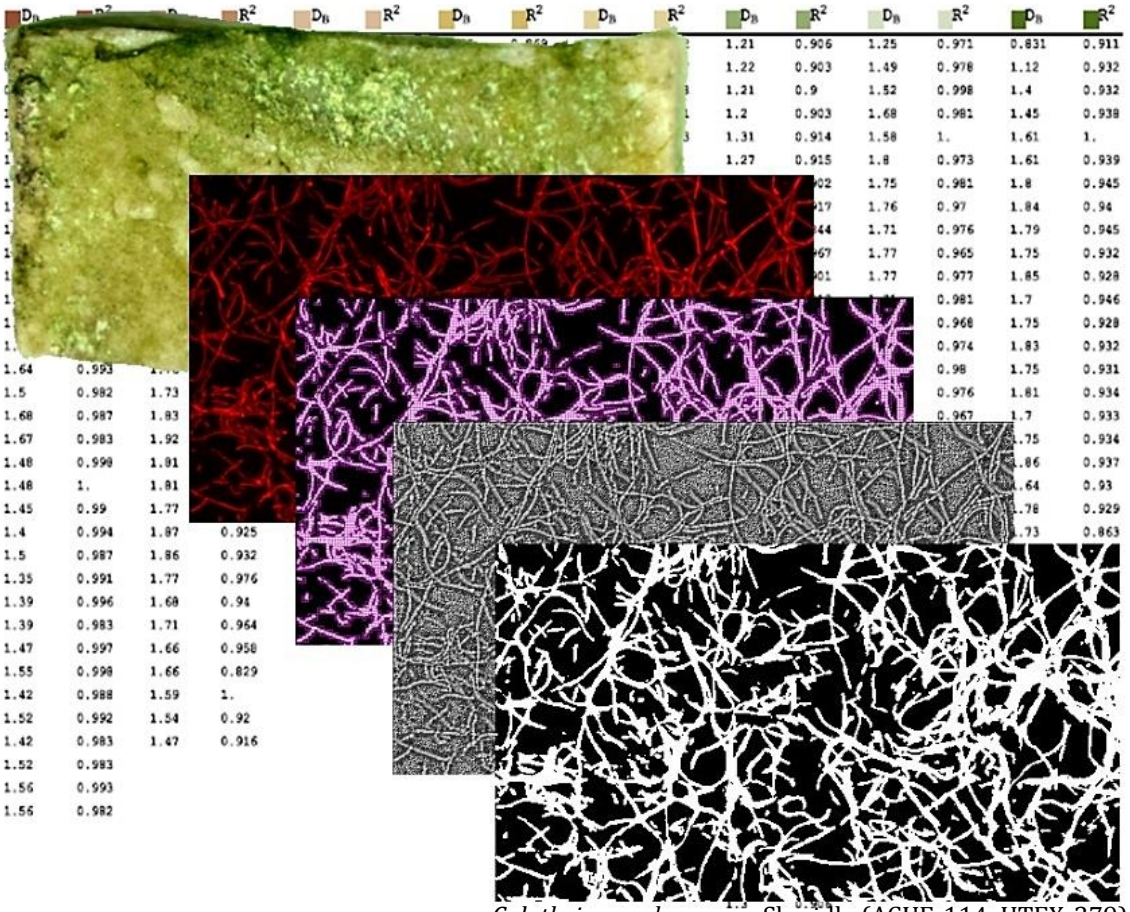
Oesterhelt, C.; Schnarrenberger, C.; Gross, W. Characterization of a sugar/polyol uptake system in the red alga Galdieria sulphuraria. European Journal of Phycology, 1999, 34(3): 271-277.

Rigano, C.; Fuggi, A.; Di Martino Rigano, V.; Aliotta G. Studies on utilization of 2-ketoglutarate, glutamate and other amino acids by the unicellular alga Cyanidium caldarium. Arch. Microbiol., 1976, 107(2): 133-138.

Xiao, R.; Zheng, Y. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnology Advances, 2016, 34(7): 1225-1244.

Società dei Naturalisti in Napoli

Working group on biofilms



Calothrix membranacea Shmidle (ACUF_114; UTEX_379)

Società dei Naturalisti in Napoli

Via Mezzocannone, 8 – Naples

www.societanaturalistinapoli.it

Email: postmaster@societanaturalistinapoli.it

ACUF

Algal **C**ollection at **U**niversity **F**ederico II

Via Cinthia, 26 - Naples

www.acuf.net