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Research Communication

A culture collection of Maltese microorganisms for application in biotechnology, biomedicine and industry

Gabrielle Zammit[*1]
[1]*Centre for Molecular Medicine and Biobanking, Biomedical Sciences Building, University of Malta*

1 Introduction

Over the years, very few studies have been conducted on microorganisms growing in the Maltese islands, and these have rarely resulted in the description of new genera, species or serovars. Two important exceptions are the studies on *Brucella melitensis*, by Sir Temi Zammit in 1905 (Wyatt, 2005) and a new serovar of *Salmonella* from Gozo (Vella & Cuschieri, 1995).

Ten years ago, sampling of microorganisms growing as biofilms on different substrates around the Maltese islands was initiated. The microorganisms consisted mainly of chemoorganotrophic bacteria, cyanobacteria and microalgae. Today the culture collection of Maltese microorganisms contains over a hundred new microbial strains that are new to science and which include freshwater, marine, soil and subaerophytic microorganisms.

The aim of the research is twofold. Firstly, it is important to characterise the Maltese microbial strains and describe new taxa as required. Secondly, the extraction of important metabolites for application in biotechnology, biomedicine and industry.

2 Materials and Methods

Sampling of microbial biofilms was carried out using non-invasive sampling techniques (Zammit, De Leo, Albertano & Urzì, 2008; Zammit, Psaila & Albertano, 2008; Zammit, De Leo, Urzì & Albertano, 2009). Isolation of microorganisms from biofilms was undertaken (Zammit, Billi, Shubert, Kaštovský & Albertano, 2011). Observations by light, confocal, scanning and transmission electron microscopy were carried out according to published methodologies (Zammit, Billi & Albertano, 2012).

Genetic studies involved the sequencing and subsequent analysis of 16S rRNA and ITS genes of cyanobacteria (Zammit et al., 2012) and chemoorganotrophic bacteria (De Leo, Iero, Zammit & Urzì, 2012) and also the 18S rRNA genes of microalgae (Zammit, Billi, Shubert et al., 2011).

3 Results and Discussion

Microbial strains in the culture collection of Maltese microorganisms are grown in a variety of nutrient media and under specific conditions of light and temperature (Zammit, Billi, Shubert et al., 2011).

Of these, the cyanobacterial strains belong to new species of the well-known genera *Leptolyngbya*, *Pseudanabaena*, *Nostoc*, *Fischerella*, *Chroococcidiopsis*, *Asterocapsa* and *Gloeocapsa*. A number of other strains belong to new genera that are currently being described. A large number of cyanobacterial strains possess a simple morphology and are considered to be members of cryptic species (Osorio-Santos et al., 2014). Some are slow growing in culture and are difficult to isolate from other microbial strains, since they are likely to share a symbiotic relationship (Fig. 1).

To date, a number of new strains of cyanobacteria from the collection have been characterised (Zammit, Kaštovský & Albertano, 2010), new genera and species have been described (Zammit et al., 2012), while the taxonomic position of others is currently being elucidated. Understanding the ecology and evolutionary history of new microorganisms is one key to realizing their potential to produce new biomolecules.

Important milestones have included the description of the new genus *Oculatella* (Zammit et al., 2012), with strains of the type species *O. subterranea*, isolated from both Maltese and Italian hypogea. This genus has since been confirmed by the description of seven other species isolated from a variety of habitats including arid to semiarid soils, a temperate lake and sea caves (Osorio-Santos et al., 2014).

O. subterranea is a filamentous cyanobacterium that

**Correspondence to*: Gabrielle Zammit (gabrielle.zammit@gmail.com)

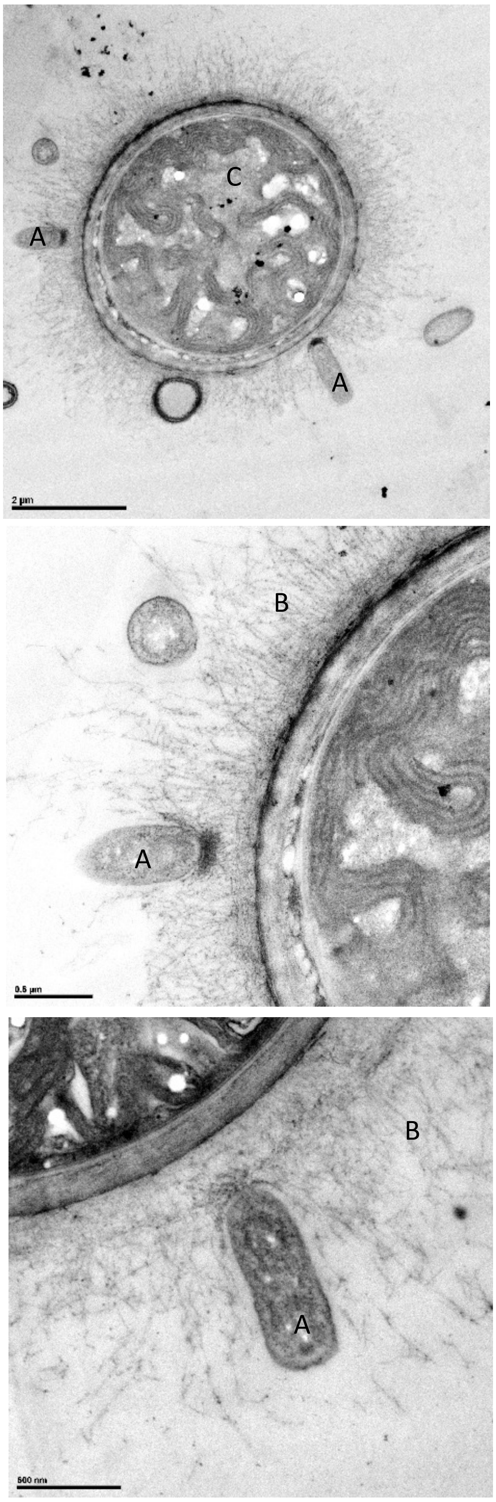

Figure 1: Transmission electron micrographs elucidate intimate symbiotic relationships. Here rod-shaped chemoorganotrophic bacterial cells (A) are seen adhering to and feeding on the capsular exopolysaccharide layer (B) produced by a larger cyanobacterial cell (C).

is characterised by fine trichomes 1–3 µm wide. These appear purple-red due to a predominance of phycoerythrin over phycocyanin inside the cells. The trichomes exhibit phototaxis and are able to glide along an extracellular sheath due to the presence of a photosensitive apical cell containing a rhodopsin-like pigment (Zammit et al., 2012).

Cytochemical stains used in transmission electron microscopy allowed detection of the presence of two different layers of carboxylic groups within the sheath. The composition of exopolysaccharides of *O. subterranea* were found to be composed of the following monosaccharides; the acidic galacturonic and glucuronic acids, and the neutral sugars; galactose, glucose, mannose, xylose, fructose and rhamnose (Bellezza, Paradossi, De Philippis & Albertano, 2003).

Apart from the important role they play in bacterial biochemistry, and the formation of a thick microbial matrix that facilitates adhesion and prevents desiccation and exposure to UV, these polysaccharides may have chemical and physico-chemical properties of industrial interest. In fact, polysaccharides released into the culture medium are anionic due to the presence of uronic acids and other charged constituents, such as sulphate groups; these latter groups may confer antiviral properties (Bellezza et al., 2003).

Lipid profiles from *O. subterranea* extracted from a limestone cave in Greece have revealed the presence of palmitic (16:0), palmitoleic (16:1), linoleic (18:2), oleic (18:1) tridecylic (13:0) and myristic (14:0) acids, isomers of C15, C17, C20 and C22 as well as stearic (18:0) and linolenic (18:3) acids (Christodoulou, Meletiou-Christou, Parmakelis, Economou-Amilli & Pantazidou, 2015). The predominance of unsaturated fatty acids is likely related to the low temperature at which the cyanobacteria were grown.

To date, little has been done to compare the fatty acid and lipid profiles from various marine, freshwater and terrestrial habitats and further studies are required in order to understand the reasons leading to the production of specific fatty acids by specific cyanobacterial strains. So far, lipids and fatty acids extracted from cyanobacterial species and used as food supplements, are characterized by the high amount of essential fatty acids such as α- and γ-linolenic acid, and their C20-derivatives, arachidonic acid and eicosapentaeonic acid.

The interesting molecular biology and biochemistry of *O. subterranea* warrant further in depth study. In fact, whole genome sequencing, together with a reconstruction of biochemical processes, are both currently ongoing and will be published in the near future.

As regards the microalgae, strains of *Trentepohlia*, *Pseudococcomyxa* and *Diadesmis* have been isolated in culture. Many other strains are characterised by prohibitive simple morphology. Genetic analysis, though, has facilitated the identification of new strains from the genus *Chlorella* (Zammit, Billi, Shubert et al., 2011) and also a recently described species, *Jenufa aeroterrestrica* (Prochazkova, Nemcova & Neustupa, 2015). *J. aeroterrestrica* strains grow on tree bark; to our knowledge this is the first time a member of this species has been isolated from a hypogean environment and successfully grown in culture.

Metabolites from a number of promising microalgal strains in the collection are currently being extracted. Our main aim is in the discovery of new bioactive substances for application in biomedicine. Other important applications of microalgal secondary metabolites are the cosmetics and aviation fuel industries. Maltese microalgae can also be used as important bioindicators for global climate variability and as important tools in forensic studies.

As with cyanobacteria, the molecules of interest include polyunsaturated fatty acids such as eicosapentaenoic acid and acrylic acid, which have anti-microbial and anti-inflammatory properties. Algal sterol extracts such as lathosterol, ergosterol, stigmasterol, 24-ethylcholesta-5,7,22-trienol, stigmasta-7,24(241)-dien-3ββ-ol, and cholesterols, have the ability to potentially lower plasma non-HDL cholesterol by increasing the excretion of total faecal sterols and decreasing cholesterol absorption and synthesis, reducing the risk of heart disease in the process. Sulfated polysaccharides have anti-inflammatory, anti-cancer and immunomodulating properties. Microalgal pigments such as lutein and astaxanthin have been shown to exhibit antioxidant, anti-inflammatory and anti-cancer activities.

Apart from the characterisation of new cyanobacterial and microalgal strains for biotechnology application, recent studies have involved the use of chemoorganotrophic bacteria in consolidating deteriorated local Globigerina limestone.

Past studies had shown that microbially induced calcite precipitation occurred naturally on ancient limestone surfaces (Zammit, Sánchez-Moral & Albertano, 2011). We exploited this phenomenon with the successful bioconsolidation of deteriorated limestone through the application of treatments enriched with *Bacillus subtilis*. The treated stone was subjected to various mechanical and physical tests in order to present a statistically robust data set to prove that the treatment was indeed effective. Uniform bioconsolidation occurred to a depth of 30 mm, an unprecedented result. Drilling resistance values for the treated limestone were similar or better than those obtained for freshly quarried stone blocks. We reported an eco-friendly bioconsolidative treatment that closely resembles the mineral composition of the limestone and that penetrates into the porous structure without affecting the limestone's natural properties. The treatment is of industrial relevance since it competes well with stone consolidants available commercially (Micallef, Vella, Sinagra & Zammit, 2016).

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References

Bellezza, S., Paradossi, G., De Philippis, R. & Albertano, P. (2003). Leptolyngbya strains from Roman hypogea: cytochemical and physico-chemical characterisation of exopolysaccharides. *Journal of Applied Phycology*, *15*(2-3), 193–200.

Christodoulou, M., Meletiou-Christou, M. S., Parmakelis, A., Economou-Amilli, A. & Pantazidou, A. (2015). Further findings from Daveli Cave (Attica, Greece) enhancing the establishment of the genus Oculatella (Pseudanabaenaceae, Cyanobacteria). *Phytotaxa*, *202*(3), 169–184.

De Leo, F., Iero, A., Zammit, G. & Urzì, C. E. (2012). Chemoorganotrophic bacteria Isolated From Biodeteriorated Surfaces In Cave And Catacombs. *International Journal of Speleology*, *41*(2), 125–136.

Micallef, R., Vella, D., Sinagra, E. & Zammit, G. (2016). Biocalcifying Bacillus subtilis cells effectively consolidate deteriorated Globigerina Limestone. *Journal of Industrial Microbiology and Biotechnology*, *43*(7), 941–952.

Osorio-Santos, K., Pietrasiak, N., Bohunická, M., Miscoe, L. H., Kováčik, L., Martin, M. P. & Johansen, J. R. (2014). Seven new species of Oculatella (Pseudanabaenales, Cyanobacteria): taxonomically recognizing cryptic diversification. *European Journal of Phycology*, *49*(4), 450–470.

Prochazkova, K., Nemcova, Y. & Neustupa, J. (2015). A new species Jenufa aeroterrestrica (Chlorophyceae incertae sedis, Viridiplantae), described from Europe. *PRESLIA*, *87*(4), 403–416.

Vella, L. & Cuschieri, P. (1995). Salmonella excretion in adult cattle on the Maltese island of Gozo. *Revue Scientifique et Technique - Office International des Épizooties*, *14*(3), 777–787.

Wyatt, H. V. (2005). How Themistocles Zammit found Malta Fever (brucellosis) to be transmitted by the milk of goats. *Journal of the Royal Society of Medicine*, *98*(10), 451–454.

Zammit, G., Billi, D. & Albertano, P. (2012). The subaerophytic cyanobacterium Oculatella subterranea (Oscillatoriales, Cyanophyceae) gen. et sp. nov.: a cytomorphological and molecular description. *European Journal of Phycology*, *47*(4), 341–354.

Zammit, G., Billi, D., Shubert, E., Kaštovský, J. & Albertano, P. (2011). The biodiversity of subaerophytic phototrophic biofilms from Maltese hypogea. *Fottea*, *11*(1), 187–201.

Zammit, G., De Leo, F., Albertano, P. & Urzì, C. (2008). A preliminary comparative study of microbial communities colonizing decorated chambers at the Hal Saflieni Hypogeum at Paola, Malta. In J. W. Lukaszewicz & P. Niemcewicz (Eds.), *11th international congress on deterioration and conservation of stone* (pp. 555–562). Torun: Nicolaus Copernicus University Press.

Zammit, G., De Leo, F., Urzì, C. & Albertano, P. (2009). A non-invasive approach to the polyphasic study of biodeteriogenic biofilms at St Agatha Crypt and Catacombs at Rabat, Malta. In *Science and cultural heritage in the mediterranean area- diagnostics, conservation, experiences and proposals for a risk map* (pp. 323–327). Palermo: Priulla SRL.

Zammit, G., Kaštovský, J. & Albertano, P. (2010). A first cytomorphological and molecular characterisation of a new Stigonematalean cyanobacterial morphotype isolated from Maltese catacombs. *Algological Studies*, *135*, 1–14.

Zammit, G., Psaila, R. & Albertano, P. (2008). An investigation into biodeterioration caused by microbial communities colonising artworks in Maltese Palaeo-Christian Catacombs. In A. Notea & Y. Shoef (Eds.), *9th international conference on non-destructive testing, microanalysis and preservation in the conservation of cultural and environmental heritage* (pp. 1–10). Jerusalem: ISAS International Seminars Ltd.

Zammit, G., Sánchez-Moral, S. & Albertano, P. (2011). Bacterially mediated mineralisation processes lead to biodeterioration of artworks in Maltese catacombs. *Science of the Total Environment*, *409*(14), 2773–2782.