

Study case on new control method of phototrophic biofilms of Collbato's Salpetre caves

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introduction

Background

Caves biofilms have been favoured by anthropogenic factors such as high **CO₂** concentration and **artificial illumination**. They involve the **biodegradation** of rocks and consequently the loss of heritage (Cervelló, 2008). Microorganisms that form the biofilm are characterized by a matrix composed primarily of exopolymers, which offers resistance. Recent studies focus on new control methods using natural biocides as an alternative to traditional active substances. It is known that some compounds isolated from marine **sponges and seaweeds** can potentially inhibit autoinducer molecules which are the responsible for quorum sensing (a very complex phenomenon of microorganism's organization) (Vasantha *et al.*, 2012) (Fig. 1).

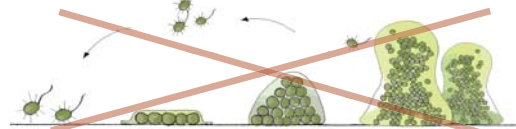


Figure 1: Control the development of a biofilm.

Study zone

It will take place in Collbato's Salpetre caves (Barcelona) (Fig. 2).



Figure 2: The situation of Collbato's Salpetre caves in Montserrat Mountain.

This project is based on the phototrophic diversity of these caves because it is the available knowledge and the cause of the current problems (Fig. 3 and 4).

Diversity species of Collbato's Salpetre caves (Roldán & Hernández-Marín, 2009):

- cyanobacteria: *Scytonema julianum*, *Nostoc punctiforme*, *Gloeocapsopsis magna* and *Leptolyngbya* sp.
- diatoms: *Diadesmis contenta*
- green algae: *Chlorella* sp., *Coccolobrya verucariae*, *Klebsormidium flaccidum* and *Myrmecia biatorellae*.



Figure 3: Cyanobacterial species on the rock.



Figure 4: Mosses and algae on the rock

Objective

verify the efficiency of three natural extracts to combat phototrophic biofilms of The Salpetre caves of Collbató

Comparing the effectiveness between them and with a chemical biocide. In addition, we need to use a hydrophobic product in order to increase the treatment efficiency and to fight the deteriorated rocks pores (Urzi & De Leo, 2007).

material and methods

The choice of natural extracts for this work has been based on existing literature such Cuzman *et al.* (2011) (Table 1). The most of them are involved in microfouling process and they should be effective against the photosynthetic microorganisms and be harmless to the rock for being applied. We propose the concentrate Algophase (A) as a chemical biocide and the Hydrophase Superfici as an hydrofobic product.

extract	source	compound active	action
Poly-alkyl-pyridinium salts (pAPS)	<i>Reniera sarai</i> (sponge)	3-alkylpyridinium	Surfactant activity towards membranes and acetylcholinesterase inhibitory activity
Zosteric acid (ZA)	<i>Zostera marina</i> (Magnoliophyta)	p-sulphoxy-cinnamic acid	Unknown: surfactant activity, quorum-sensing inhibitors.
The extract of <i>Ceramium botryocarpum</i> (CBE)	<i>Ceramium botryocarpum</i> (Rhodophyta)	dichloromethane	Unknown: allelopathically.

Table 1: Characteristics used extracts.

Ex situ experiment

- 4 Petri dishes/medium (x3) (Fig. 5)
- Cyanobacteria: 57x105 cells/mL; BG11 medium
- Green algae: 38x105 cells/mL; modified BG11 medium
- Cylindres with extract in different concentrations (w/v%)
- Constant conditions

Visual evaluation at 7, 24 and 75 days.

- Determination of Minimum Inhibitory Concentration

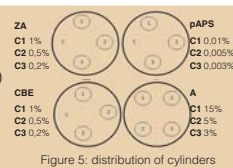


Figure 5: distribution of cylinders used in the experiment.

In situ experiment

5 Circles on the rock (Fig. 6)

- Cleaning
- Extract applications
- Optical evaluation: the covered area (%)
- Molecular evaluation: ARISA fingerprint
- Denaturing Gradient Gel Electrophoresis (Akatoeva *et al.*, 2009)
- Colour test of the rock

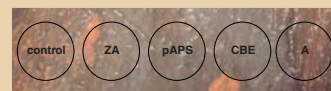


Figure 6: distribution of the circles on the rock

expected results

Ex situ experiment

The results of the experiment will be classified as: + = efficient; - = no efficient; +/- = uncertain (Table 2).

- The most of the extracts will be efficient against cyanobacteria rather than algae.
- Probably, the better natural product will be pAPS.

	concentration	ZA	pAPS	CBE	A
Algae	C1	+/-	+/-	+/-	+
	C2	+/-	+/-	+	+
	C3	+/-	+/-	+	+
Cyanobacteria	C1	+	+	+/-	+
	C2	+	+	+/-	+
	C3	+	+	+	+

Table 2: The results according to the products and their concentrations (w/v %).

pAPS could be used as an efficient biocide, especially for cyanobacteria.

They have an inhibitory effect in low concentration and show less colour change of the rock.

In situ experiment

- The circle of less covered area would be pAPS (Fig. 7).
- In addition, the circle of pAPS will show less colour change of the rock.

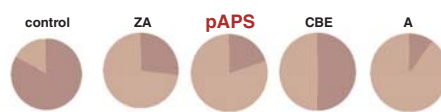


Figure 7: representation of the covered areas by microorganisms (%)

- DGGE analysis: it allows comparison the composition of species of the treatments with the control, this is needed because the extracts could be used as a substrate for other microorganisms and favour them.

conclusion

It would be suitable to do a more complex study using molecular techniques at the beginning.

In the Salpetre caves of Collbató are needed:

- **Preventive measures: reducing lamps and the number of people for visit. And also, proposing the use of flashlights inside the caves.**
- **Corrective measures: pAPS treatment.**

We have to take into account that:

- the mechanical abrasion could damage the rock and also the proliferation of some microorganisms may be dispersed throughout the cave.
- it could generate a fungal proliferation

Future research is necessary!

dissemination plan

- Inform the problematic and get grants: the Council of Collbató, Culture Department of *Generalitat de Catalunya*, *Diputació de Barcelona*, *Fondo Europeo de Desarrollo Regional*.
- Social consciousness: spreading on the news and writing a leaflet.
- Collaboration: work with *Escola Superior de Conservació i Restauració de Béns Culturals de Catalunya* and *Instituto del Patrimonio Cultural de España*.
- Future publications:
 - International Journal of Speleology
 - Geomicrobiology Journal
 - Journal of Bioremediation & Biodegradation

"It produces an immense sadness to think that nature speaks, while mankind does not listen"

Victor Hugo

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