

Supplementary Materials

# Early Detection of Phototrophic Biofilms in the Polychrome Panel, El Castillo Cave, Spain

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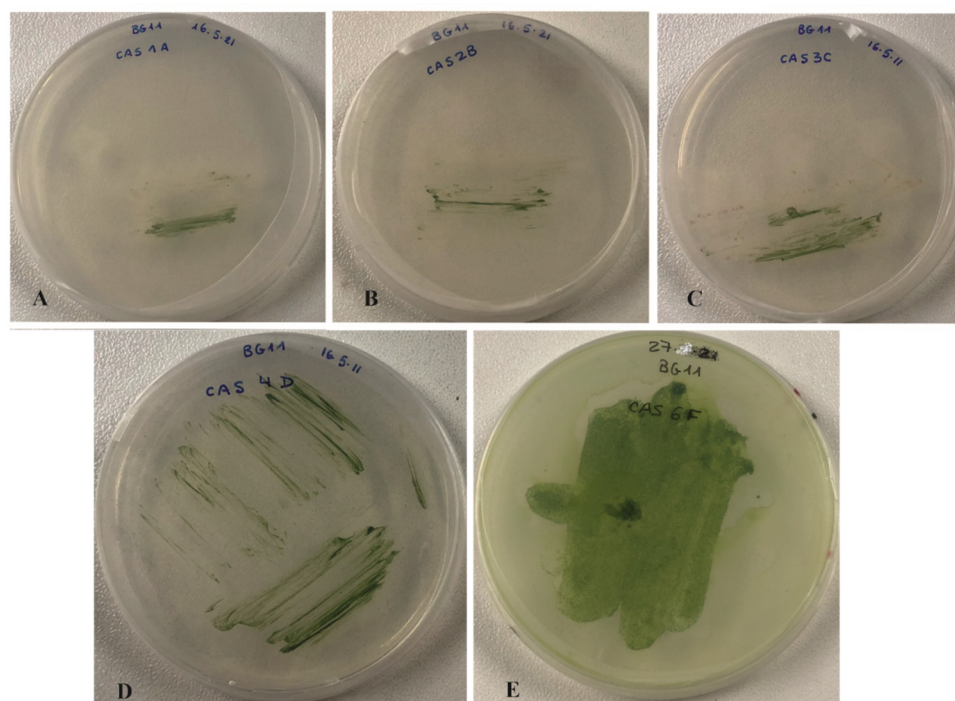
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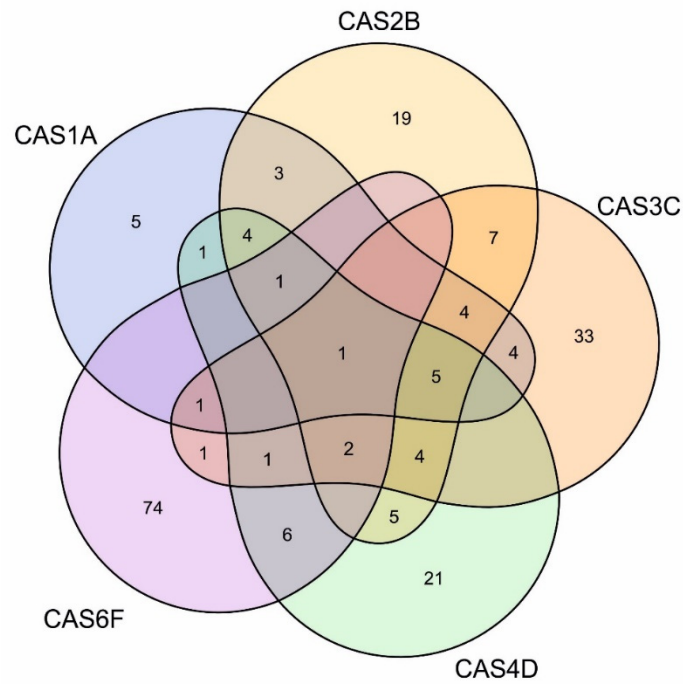
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**Figure S1.** Samples grown in BG11 medium. **A:** Sample CAS1A. **B:** Sample CAS2B. **C:** Sample CAS3C. **D:** Sample CAS4D. **E:** Sample CAS6F.



**Figure S2.** Prokaryotic community distribution in El Castillo Cave. Venn diagram showing the overlap between the ASVs generated by the five samples.

**Table S1.** Monthly data on visits to the cave (total, average and maximum daily visits) compared with the mean daily variations of temperature and CO<sub>2</sub> concentration for cave air.

Month/Year	Visitors			Mean Daily Variations (with Visitors)	
	Total	Daily avg	Daily max.	T (°C·day <sup>-1</sup> )	CO <sub>2</sub> (ppm·day <sup>-1</sup> )
10/2020	1621	60	115	0.101	68
11/2020	184	14	67	0.050	68
12/2020	419	28	70	0.065	59
01/2021	493	21	51	0.045	49
02/2021	756	33	84	0.067	57
03/2021	887	35	114	0.069	-
04/2021	1529	59	111	0.070	57
05/2021	1382	53	113	0.019	55
06/2021	2195	84	129	0.115	112
07/2021	3212	115	136	0.112	153
08/2021	3183	118	145	0.125	118
09/2021	2667	111	186	0.124	142
<b>Annual</b>	<b>18528</b>	<b>61</b>	<b>110</b>	<b>0.083</b>	<b>85</b>
Days without visitors (yearly avg):				0.019	72

**Table S2.** Description of samples from the Polychrome Panel, El Castillo Cave.

Sample	Location and Description of the Samples
E1A	Faint green stains in Polychrome Panel (Figure 2A,B)
E2B	Faint green stain in Polychrome Panel (Figure 2C)
E3C	Faint green stain in Polychrome Panel
E4D	Faint green stain in Polychrome Panel
E6F	Abundant biofilm in a spotlight away from Polychrome Panel (Figure 2D)

**Table S3.** DNA concentration of samples from the Polychrome Panel, El Castillo Cave.

Sample	Concentration (ng / $\mu$ L)
E1A	< 0.5
E2B	< 0.5
E3C	< 0.5
E4D	< 0.5
E6F	< 0.5
CAS1A	332.0
CAS2B	69.8
CAS3C	306.0
CAS4D	119.0
CAS6F	167.0

**Table S4.** Number of ASVs, taxon richness and  $\alpha$ -diversity indexes for El Castillo Cave.

Sample	ASV	Faith-pd	Shannon	Evenness
CAS1A	29	3.74	3.54	0.73
CAS2B	55	4.53	4.23	0.73
CAS3C	63	6.68	4.63	0.77
CAS4D	51	4.00	3.70	0.65
CAS6F	87	5.72	4.06	0.63

**Table S5.** Prokaryotic ASVs distribution in El Castillo Cave.

Domain	CAS1A	CAS2B	CAS3C	CAS4D	CAS6F
<i>Bacteria</i> %	99.9	100.0	100.0	99.9	100.0
Unassigned %	0.1	0.0	0.0	0.1	0.0

**Table S6.** Eukaryotic ASVs distribution in El Castillo Cave.

Domain	CAS1A	CAS2B	CAS3C	CAS4D	CAS6F
<i>Eukaryota</i> %	80.8	98.6	84.6	100.0	100.0
Unassigned %	19.2	1.4	15.4	0.0	0.0