CYANOCHIP2.0 FOR IN SITU SEARCHING FOR CYANOBACTERIAL BIOMARKERS IN PLANETARY EXPLORATION AND ENVIRONMENTAL MONITORING. Y. Blanco[1], A. Ianneo[1,2], J. Aguirre [3], D. Billi[2] and V. Parro[1], [1]Centro de Astrobiología (INTA-CSIC), Carretera de Ajalvir km4, Torrejón de Ardoz, Madrid, Spain (blancoly@cab.inta-csic.es),[2]University of Rome Tor Vergata, Via della Ricerca Scientifica snc, Rome, Italy,[3]Centro Nacional de Biotecnología (CSIC), c/ Darwin 3, Madrid, Spain.

Introduction: Cyanobacteria are among the most ancient life forms on Earth [1]. They are highly versatile in their metabolism and have the ability of thriving in in a wide variety of habitats from open water, to on, inside or underneath the rocks, even in the middle of salt crusts [2]. Evidences of cyanobacteria remains have been dated at >3.5 Ga and it could be argued that similar forms of live might have reach Mars during the late heavy bombardment [3] and had the opportunity to colonize the planet. Therefore, extant or extinct cyanobacterial biomarkers are priority targets in the searching for life on Mars. In order to bypass the destructive effect of thermal volatilization on sample preparation [4], we developed a mild and non-destructive bio-affinity-based method. The CYANOCHIP [5], is an immunosensor to detect cyanobacteria with taxonomical resolution. We expanded the CYANOCHIP with four new, redundant, antibodies to natural isolates the desert loving *Chroococcidipsis* genus, and validated it with environmental samples from terrestrial analogues.

Methods: Two new anhydrobiotic cyanobacterial strains, *Chroococcidiopsis* sp. CCMEE 029 and *Chroococcidiopsis* sp. CCMEE 057 isolated from Negev and Sinai deserts respectively, were used as immunogens to produce 4 polyclonal antibodies, which were included in CYANOCHIP to achieve the expanded version named CYANOCHIP2.0. Antibodies were purified, fluorescently labeled and titrated. Antibodies were printed on epoxy-activated glass slides and tested one by one by fluorescent sandwich microarray immunoassays (FSMI) using each antigen/antibody pair to disentangle cross reactivity events. To validate CYANOCHIP2.0, three microbial mats from Antarctica were analyzed by FSMI. They were collected in Deception Island, Byers Peninsula (Livingston Island) and McMurdo Ice Shelf.

Results: The new antibodies were titrated by FSMI by using a constant immunogen concentration. The working dilutions corresponded to 4 µg/mL for the strain O29 and 1 µg/mL for the strain O57. The limit of detection (LOD) of three of them by FSMI was 10^2-10^3 cells/mL, while in the case of the antibody to strain O29 grown in solid BG11 medium, the limit of detection was very low (10^5-10^6 cells/mL). The specificity of each antibody was assayed by cross-reactivity analysis through FSMI by using each cognate immunogen and the corresponding fluorescent antibody as tracer. The fluorescence intensity of the corresponding spots of all the antibodies on the microarray in each assay was quantified and expressed as an antibody graph. The results showed, as expected, strong crossreactivity between the antibodies to *Chroococcidiopsis* spp. Then, the CYANOCHIP2.0 was assayed by FSMI using environmental extracts prepared from Antarctic microbial mats and a mixture of 21 fluorescent cyanobacterial antibodies as tracers. Multiplex assays showed high fluorescent positive reactions with antibodies to benthic species isolated from Antarctica (*Anabaena* sp. and *Leptolyngbya* sp.) in a sample from Byers Peninsula and only *Anabaena* sp. in a *mummified* (c.a. 1000 y old) mat collected at McMurdo Ice Shelf. Also, high positive signals were detected with antibodies to planktonic species (*Aphanizomenon* sp.) in the samples from Byers and Cerro Caliente, as well as high positive signals of *Microcystis* spp. *(mostly M. aeruginosa*) in the mummified sample. Minor intensity but still positive signals were detected in antibodies against *Chroococcidiopsis* sp. Fluorescent microscopy and DNA sequencing confirmed the presence of cyanobacterial cell morphologies (filaments and clusters) and sequences in the samples under study, except in the mummified sample were no DNA sequences from cyanobacteria were retrieved.

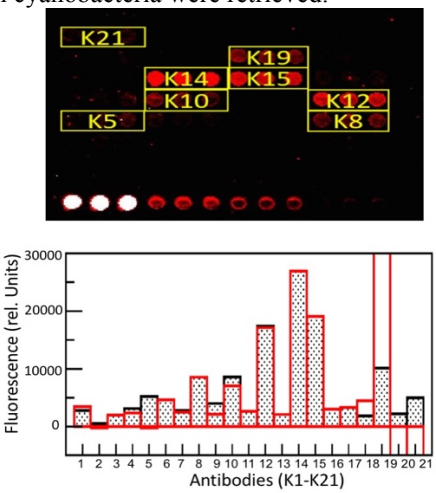

Figure 1. Fluorescence image of CYANOCHIP2.0 obtained after FSMI of an Antarctic microbial mat from Byers peninsula (top) and the quantification and deconvolution analysis (bottom).

References: [1] B. E. Schirrmeister et al. (2016) *Int. J. Astrobiology* 15, 187–204, [2] J. Wierzchos et al. (2006) *Astrobiology* 6, 415-422, [3] M. Maurette et al. (2006) *Adv. Space Res*., 38, 701–708, [4] H. Steininger et al. (2012) *Planet. Space Sci.* 71, 9-17, [5] Blanco, Y., *et al.* (2015) *Environ. Sci. Tech.* 49, 1611-20.

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