



## **Polyphasic approach to characterize heterotrophic bacteria of biofilms and patina on walls of the Suburban Bath of the Herculaneum's archaeological excavations in Italy**

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Built between the walls of Herculaneum excavations, one of the world's most important archaeological sites, and the sea in the early 1st cent. AD, the Suburban Bath is one of the best thermal complexes better preserved in ancient times. The entrance opens onto a large courtyard that leads into a hallway well lit by a skylight, impluvium, with a portrait of "Apollo". From this room you can access various parts of the *thermae*, all beautifully preserved. A single room, mostly occupied by the pool, serving both *apodyterium* (dressing room) that *frigidarium*. Among *tepidarium* and *frigidarium* there's a room elegantly decorated with stucco and marble. The vestibule opens to the right, through a corridor, onto a waiting room with a floor in *signinum opus* and into a *praefurnium* (oven for heating). A large pool of *tepidarium*, connected with *laconicum*, a small circular room for the baths sweat, is also present. The *calidarium*, as usual, has a small tank for hot water and a basin for washing in cold water. Behind the *calidarium* is the *praefurnium*, an environment with the boiler for heating the bath. Although the suburban baths are well preserved, unfortunately in you can observe the development of visible microbial coatings. During the biodeterioration process, secondary colonization of wall is due to heterotrophic bacteria and fungi that induce deterioration cause structural as well as aesthetic damage such as the discoloration of materials, the formation of crusts on surfaces and the loss of material.

This investigation was carried out sampling the surfaces of walls of different rooms in the Suburban *Thermae* according to Italian legal procedures. Depending on the samples typology, sampling was carry out using sterile nitrocellulose membranes pressed on the surface of the walls, sterile swabs or with sterile tweezers by tearing out surface material. The samples were suspended in physiological solution and immediately refrigerated until analysis. Isolated colonies grown on PCA plates were purified in the same growth medium by streaking and differentiated by assessing their morphological (phase-contrast microscopy) and biochemical characteristics (Gram-stains KOH-lysis and catalase activity). Cultural-based method allow us to identify by 16S and 26S rRNA partial sequence analysis, heterotrophic bacteria belonging to different genera as *Bacillus*, *Pseudomonas*, *Aeromonas* and *Microbacterium*. By using this approach, *Bacillus*-related species (*B. benzoevorans*, *B. megaterium* and *B. pumilis* and *B. megaterium/B. simplex* group) as well as *Aeromonas sobria/Aeromonas salmonicida/Aeromonas hydrophila* group, *Pseudomonas plecoglossicida* and *Microbacterium esteraromaticum* were isolated in different sample points analysed. DGGE analysis of PCR amplified V3 region of rDNA from DNA directly recovered from samples of biofilms and patina, enabled identification of bacterial species not found using culturable technology, as those closest related to *Aeromonas*, *Paenibacillus*, *Brevibacterium*, *Exiguobacterium*, *Microbacterium*, *Brevibacterium*, *Stenothophomonas* and *Streptomyces*. Combination of culture-dependent and independent methods provide a better characterization of heterotrophic microbiota that colonize the surface of ancient decorated walls and can contribute to understand the potential of biodeterioration activity by heterotrophic microorganisms.