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Surface enhanced Raman spectroscopy and cultural heritage biodeterioration: Fungi identification in earthen architecture from Paraíba Valley (São Paulo, Brazil)

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ABSTRACT

In this work, Surface Enhanced Raman Spectroscopy (SERS) was employed in the taxonomic identification of fungi found in biofilms formed on earthen architecture walls (adobe, wattle and daub, and rammed earth) of historical buildings in the region known as Paraíba Valley (or São Paulo Historical Valley), which are representative of the first phase of the Brazilian coffee cycle (1820–1880). Very few studies are reported in the literature where SERS-based techniques are used in fungi identification, most of them focused on clinical diagnosis. In the present investigation, pure colonies isolated from biofilms on earthen walls previously identified by classic taxonomy and molecular biology were selected. The genera were *Trichoderma*, *Cladosporium*, *Aspergillus*, *Neurospora*, *Fusarium* and *Penicillium*. The fungi were cultured on solid potato dextrose agar, extracted with ethyl acetate and the extracts were applied on dried Au nanoparticles. The SERS spectra exhibited bands in the 600–1800 cm^{-1} region which are characteristic of each genus, except *Penicillium*, as revealed by PCA statistical analysis. This work reports the use of a facile to prepare SERS-active substrate in the identification of microbial communities on earthen architecture walls and is the first step of an investigation aiming at the fast identification of fungi species from biofilms formed on earthen architecture buildings without the need of isolating the pure cultures.

1. Introduction

Filamentous fungi play an important role in biotechnology and also as biodeterioration agents. The methods used in their identification were originally only at the biological level, as in classical taxonomy, through the observation of their macro and micro morphological characteristics. Later, molecular biology allowed DNA base pairs sequencing and, more recently, mass spectrometry hyphenated techniques and infrared absorption spectroscopy were introduced, aiming at to identify the chemical signatures which could be used as chemotaxonomic markers [1].

Another approach involves chemical speciation coupled to high spatial resolution and cytoplasm spectrochemical characterization, which can lead to the understanding of the hyphae growing processes. Prusinkiewicz et al. adopted such approach and used FTIR, Raman spectroscopy and Surface Enhanced Raman Spectroscopy (SERS) to obtain information on *Aspergillus nidulans* and *Curvularia protuberata* biochemistry. In the SERS measurements, Au nanoparticles were synthesized by adding $HAuCl_4$ directly to the culture media containing *A. nidulans* [2].

The literature on microorganisms identification by Raman spectroscopy was recently reviewed [3] and the authors compiled a wide range of applications, including medical diagnosis [4], which benefited from the fingerprinting characteristics of the technique for both whole-organism [5] or metabolites [6]. Raman spectroscopy was used, for example, in the differentiation of taxons from distinct macromycetes genera, based on the lipid and protein signatures observed in the spectra collected from basidiospores [7]. The authors used linear discrimination to assign 90% of the spectral features to the correct genus but it was not possible to achieve the identification at the species level.

The capability of Raman microscopy to allow a fast characterization and identification of individual spores of a variety of micromycetes species was demonstrated by Goshal et al. [8], where the authors compiled a reference library of Raman spectra of micromycetes

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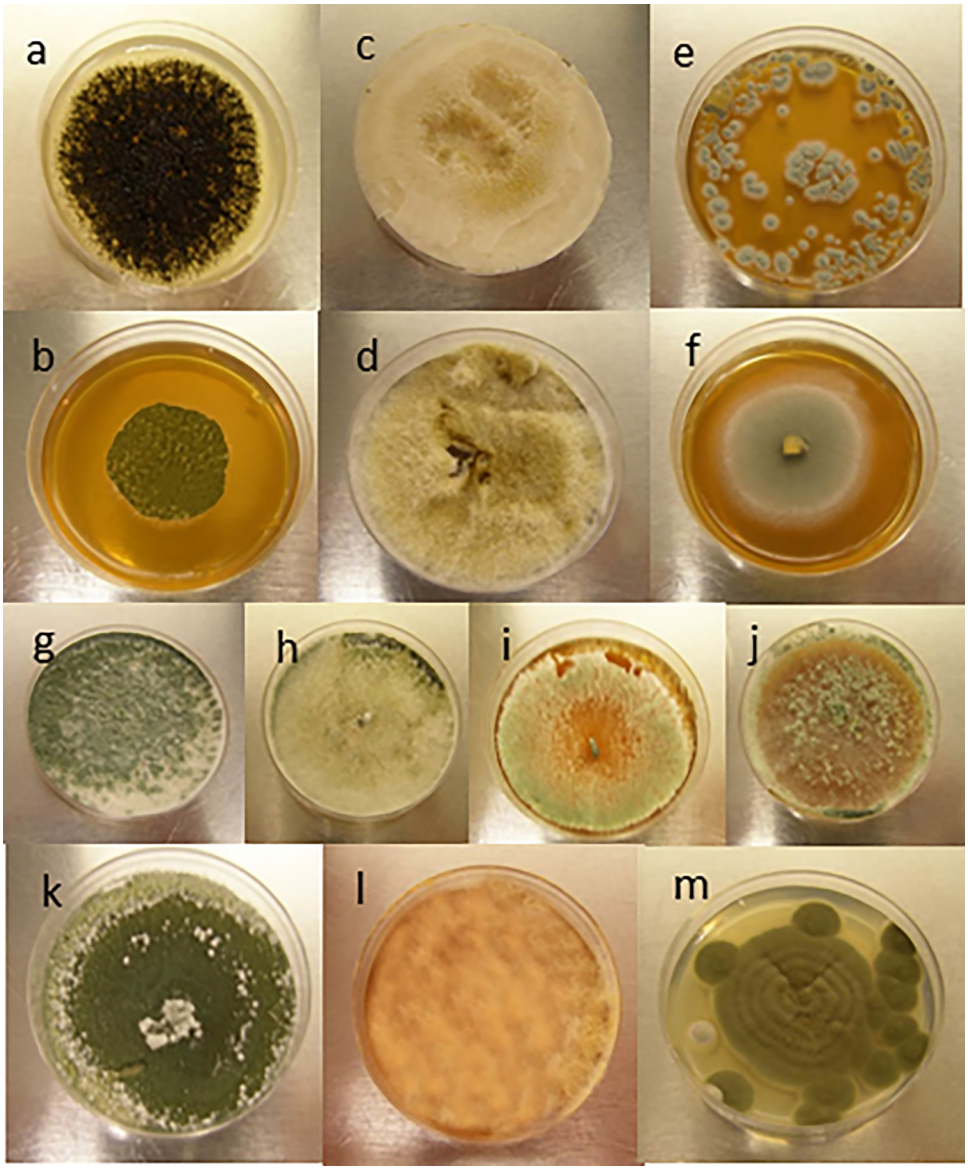

Fig. 1. Fungi isolate used in the SERS measurements: a) *Aspergillus carbonarius*; b) *A. parasiticus*; c) *Fusarium aff. incarnatum*; d) *F. equiseti*; e) *Penicillium aff. flavigenum*; f) *P. chrysogenum*; g) *Trichoderma atroviride*; h) *T. koningiopsis;* i) *T. aff. atroviride*; j) *T. harzianum*; k) *T. longibrachiatum*; l) *Neurospora sitophila*; m) *Cladosporium uredinicola*.

typically associated with humid environments. The spatial resolution in Raman microscopes is diffraction limited to the micrometric level, which is enough to the investigation of individual cells with the further advantages that minimal sample preparation is required and small volumes are employed.

Among other plasmonic techniques, Surface Enhanced Raman Spectroscopy (SERS) based techniques [9] are becoming tools of choice in biomedical studies [10]. The very large enhancement in the intensity of selected bands in the Raman spectra (10^4–10^8) originates mostly from the amplification of the electric field of both incident and scattered electromagnetic radiation, in resonance with localized surface plasmon (LSP) absorption associated with nanostructured metal surfaces or particles (typically Ag, Au and Cu) [11].

Regarding the use of SERS in studies focused on fungi or fungi metabolites, Szeghalmi et al. [12] reported on the growth of fungal hyphae (*Aspergillus nidulans*) on commercial nanostructured gold substrate, highlighting the potential of SERS in life sciences. The same type of SERS active substrate was employed by He et al. [13] to discriminate among five bacillus spores (*B. cereus* ATCC13061, *B. cereus* ATCC 10876, *B. cereus* sp., *B. subtilis* sp. and *B. stearothermophilus sp.*); SERS was used to enhance the Raman signal from the spores and PCA was applied considering the 900–1200 cm^{-1} spectral window. Martin-Sanchez et al. [14] used silver nanoparticles to investigate the black stains formed in Lascaux Cave and found them to be melanine from the fungus *Ochroconis* sp. Functionalized Ag nanoparticles were synthetized to target *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Aspergillus fumigatus* [15] and, in recent papers, SERS spectra were processed using chemometrics in the characterization of clinically relevant fungi (*Aspergillus fumigatus ss.*, *A. fumigatus* complex species and *Rhizomucor pusillus*) within a clinical diagnosis perspective [16] and in dermatophyte fungi identification at genus and species-level [17].

As it can be concluded from some investigations cited above, the Raman spectra reported in microorganism studies present a complex structure, and their interpretation is frequently made using chemometrics. Multivariate exploratory methods such as Principal Component Analysis (PCA) are among the most important and popular chemometric tools for performing an easier visualization of data trends or patterns and for finding outliers. Briefly, this method performs a