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Cladosporium cladosporioides (strain Clc/1): a candidate for low-density polyethylene degradation

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Abstract

Background Plastic is one of the most widely used materials worldwide in various fields, including packaging and agriculture. Its large quantities require proper disposal and for this reason more and more attention is paid to the issue of degrading plastic. Thanks to the production of non-specific enzymes, fungi are able to attack complex and recalcitrant xenobiotics such as plastics. In recent years, several spectroscopic methods were used to study the plastic degradation ability of different fungal species. Among these, Fourier transform infrared (FT-IR) and FT-Raman spectroscopy techniques are the most used. Surface-enhanced Raman scattering (SERS) spectroscopy is a powerful technique which uses metal nanoparticles (NPs) to enhance the Raman signal of molecules adsorbed on the NPs surface. In this work, the isolation of different fungi from field-collected plastic debris and the ability of these isolates to growth and colonizing the low-density polyethylene (LDPE) were explored by using scanning electron microscope (SEM), attenuated total reflectance-Fourier transform infrared (ATR-FTIR) and SERS spectroscopies.

Results Forty-seven fungal isolates belonging to 10 genera were obtained; among them only 11 were able to grow and colonize the LDPE film. However, after 90 days trial, only one isolate of *Cladosporium cladosporioides* (Clc/1) was able to carry out the initial degradation of the LDPE film. In particular, based on SEM observations, small cavities and depressed areas of circular shape were visible in the treated samples. Additionally, ATR-FTIR, normal Raman and SERS analyses supported the structural changes observed via SEM. Notably, ATR-FTIR and normal Raman spectra showed a significant decrease in the relative intensity of the methylene group bands. Similarly, the SERS spectra of LDPE after the fungal attack, confirmed the decrease of methylene groups bands and the appearance of other bands referring to LDPE polyphenolic admixtures.

Conclusions These results suggest that *Cladosporium cladosporioides* Clc/1 is able to carry out an initial degradation of LDPE. Moreover, combining ATR-FTIR, Raman and SERS spectroscopies with SEM observations, the early stages of LDPE degradation can be explored without any sample pretreatment.

Keywords *Cladosporium cladosporioides*, LDPE, Vibrational spectroscopy, Plastic degradation, SERS

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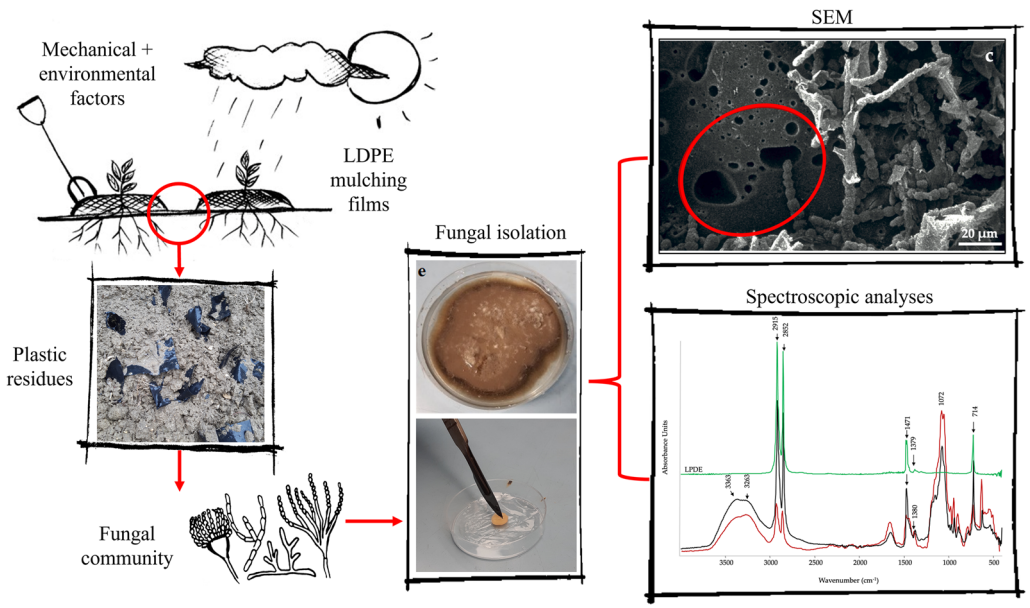
Graphical Abstract



Introduction

Plastics are represented by a wide range of synthetic or semi-synthetic materials, obtained from oil and natural gas, which under certain conditions of temperature and pressure, undergo permanent variations in shape [1]. Owing to their physico-chemical characteristics, these materials tend to be exceptionally stable and durable. However, these qualities make them very persistent in the environment and hard to be degraded [2].

World plastic production is estimated to be around 400 million tons per year and of these, approximately 90% are plastic produced from fossil sources [3]. Far from declining, the annual growth rate of the plastics market production is estimated to be 4% at least until 2025 [3]. In 2021, 57.2 million tons of plastic were produced in Europe. Of these, 86.6% were fossil-based, 10.1% were post-consumer recycled plastics, and only 2.3% were represented by bio-based plastics [4]. Regarding post-consumer plastic waste management in 2020 in Europe, data shows that a consistent part of it was used to produce energy (42%) or recycled (35%) and only 23% was accumulated in landfills [4]. Although the fraction of plastics destined for energy recovery through waste-to-energy plants and recycling has increased significantly, the accumulation of plastics still affects many habitats: oceans, seas, rivers, lakes, soil, air, ice [5] and living organisms [6], and in human placenta as well [7].

Among plastic, polyethylene (PE) is one of the most widespread and widely used synthetic material in the world [8]; it is mainly used to produce plastic films, shopping bags and food packaging, and account for up to 64% of single-use plastics that is discarded and accumulated in the environment after a short period of usage [9, 10].

Nowadays, low-density polyethylene (LDPE) mulch films account for more than 40% of the total plastic material used in agriculture [11]. These materials help to enhance the water use efficiency of the crop [12], reduce weeds growth [13, 14] and the use of herbicides [15]. In addition, they increase soil temperature and humidity [16] so that preventing the soil erosion, and consequently improving the crop yield and quality [17]. Extensive use of LDPE films and mismanagement in disposal has generated a high amount of residues in soil, amounting to about 72–259 kg/ha [18]. The natural degradation rate of these residues is extremely slow; consequently, they accumulate in all environmental compartments. Specifically, plastic residues through mechanical abrasion, mechanical friction, thermal and ultraviolet decomposition, photooxidation, and biodegradation [19–21] are degraded into microplastics with sizes larger than 1 µm and smaller than 5 mm and nanoplastics with dimensions smaller than 100 nm [22–25]. The presence of micro and nanoplastics raises particular concerns about their still poorly understood impact on the soil–plant system and agroecosystems. A

great deal of knowledge is required to understand the possible physical, chemical, and biological changes of plastic residues in order to assess their effects on living organisms. Therefore, developing effective processes to accelerate the degradation rate of plastics is critical to avoid accumulation phenomena. However, all methods involving the chemical–physical approaches [19] imply high costs and energy consumption for their implementation. Accordingly, several studies are attempting to investigate biodegradation methods, representing an eco-friendly approach for plastic waste processing [26, 27].

Several microbial species capable of degrading plastics have been identified [26]. Frequently, microbiomes operate in conjunction with abiotic agents (i.e., heat and light) to influence the structural integrity of polymers and to make them more accessible to enzymatic attack [26]. Therefore, the identification of microorganisms capable of breaking down plastic residues is an eco-sustainable strategy.

Several studies have emphasized the contribution of fungi in plastic degradation under laboratory conditions due to their ability to produce a large number of enzymes [28, 29]. In particular, several Ascomycota, such as *Aspergillus* spp. [30–45], *Cladosporium cladosporioides* (Fresen.) G.A. de Vries [38], *Fusarium* spp. [33, 36, 37, 39, 46, 47] and *Penicillium* spp. [32, 33, 38, 48–50] as well as different Basidiomycota [51, 52] and Zygomycota [33, 36] have shown to be capable of degrading different petroleum-based plastics. Concerning the activity of fungal enzymes involved in PE degradation, peroxidase, oxidase, cutinase and lipase seem to play a crucial role in this process [53, 54]. The initial degradation process of PE chains could be mediated by both enzymatic and abiotic factors; moreover, once PE has reached a chain length of 10 to 50 carbon atoms, it may be degraded enzymatically through the hydrocarbon metabolic pathway [55]. The long-chain polymer structure and hydrophobicity might prevent the hyphae adhesion on the surface of the polymer. Fungi, however, have developed adaptation strategies through the production of hydrophobins, small wall proteins responsible for the adhesion of hypha to the plastic material [54].

Fourier transform infrared (FT-IR) and Raman spectroscopies are the most widely used analytical techniques to identify plastics in the environment [56], due to the advantage of requiring no sample pretreatment, as well as high sensitivity and reproducibility [57]. These spectroscopic techniques can be combined with scanning electron microscopy (SEM), which can provide details on the structure and surface morphology of plastic residues, such as the degradation of PE by ascomycetes [54], and thus producing high-resolution images of the analyzed surface [57].

Surface-enhanced Raman scattering (SERS) is a technique widely applied to detect low concentrations of chemical substances such as pollutants, or plastic microparticles [58–60]. With the discovery of SERS in 1974, considerable interest was attracted due to the strong enhancement of the weak Raman signal thanks to the use of nanostructured metals (Au and Ag) [61]. The interaction of radiation with nanostructured metals induces localized surface plasmon resonance, which provides a strong increase in Raman scattering of up to 10^{14} orders of magnitude with respect to the normal Raman technique [62]. Additionally, the enhancement of the signal depends on the optical properties of the nanoparticles (NPs) (composition, size, and shape), the laser excitation characteristics, as well as the molecular characteristics and its Raman cross sections [63, 64]. In spite of the widely recognized advantages of the SERS technique, no reference has been found on the SERS analysis of plastic degradation by fungi.

The aim of this work was to test the ability of different fungal isolates from plastic debris collected in two different agricultural fields to grow and degrade LDPE on a laboratory scale. LDPE degradation was assessed by using SEM combined with ATR-FTIR, Raman and SERS spectroscopic techniques.

Materials and methods

Sampling, mycelial isolation and molecular analyses

Samplings were carried out during June and August 2020, in two agricultural fields located in the province of Rimini and Bologna (Emilia-Romagna, Italy). Plastic debris of around 2–5 cm^2, soft and thin (less than 0.3 mm) were collected from the soil and stored in sterile plastic containers, in the dark at room temperature. The fragments were thereafter characterized by using ATR-FTIR spectroscopy in order to identify and select LDPE plastic.

Mycelial isolations were carried out from 30 LDPE plastic debris (15 for each field) the same day of sampling by placing a cut of each plastic fragment (5 × 5 mm) on Petri dishes previously filled with potato dextrose agar (PDA, 20 g/L) medium supplemented with chloramphenicol and streptomycin. For each plastic sample five Petri dishes were made.

After 24 h, under a stereomicroscope, the single small colonies originated on the surface of the plastic fragments were transplanted into new Petri dishes.

Mycelial pure cultures were morphologically assigned to a fungal genus by using taxonomic keys [65].

Molecular analyses were carried out by amplification of the internal transcribed spacer (ITS) region of the rDNA using primer pairs ITS1F/ITS4 [66, 67] in a direct

approach which avoid DNA extraction [68]. Direct polymerase chain reactions (PCRs) were carried out in 50 µL reaction volumes using 2×BiomixTM (Bioline) and amplifications were run in a SimpliAmp thermal cycler (ThermoFisher) as follows: 95 ℃ for 6 min, followed by 30 cycles of 94 ℃ for 30 s, 56 ℃ for 30 s. PCR products were purified using NucleoSpin® Gel and PCR Clean-up kit (Macherey–Nagel, Düren, Germany) and sequencing was performed with both ITS forward and reverse. Sequences generated in this work were compared with existing sequences in the GenBank using BLASTn considering for species identification an identity greater than or equal to 98% and deposited in GenBank.

Plastic degradation assay

For the 90 days plastic degradation assay, an LDPE film (6 µm thick, 6 g/m^2) was cut into discs about 5.5 cm in diameter and sterilized at 121 ℃ for 20 min. After sterilization, the LDPE discs were inserted inside sterile Petri dishes 6 cm in diameter. Each disc was wetted with 300 µL of a liquid mineral salts media devoid of any carbon source as reported by Das and Kumar [39]. The prepared Petri dishes were than inoculated with a 0.6-cm-diameter plug of 15 days old colony and five replicates were made for each isolated species. One week after inoculation, the agar plug from each plate was removed and every 10 days 50 µL of mineral salt medium were administered to the colonies. At the end of the trials, the plates were open, and the LDPE disks were freeze-dried in a Virtis Benchtop 2K lyophilizer (SP Industries, Warminster, Pennsylvania) for further investigations.

SEM analyses

SEM microscopy was carried out in order to verify the adhesion and growth of the fungal hyphae and the degradation of the plastic substrate in comparison to an untreated (autoclaved but not inoculated) one. A small piece of LDPE disk of 1 cm^2 of each species was washed and firstly fixed in 5% glutaraldehyde solution for 24 h at 4 ℃. Subsequently, 2 washes of 7 min each in 0.1 M phosphate buffer and a series of washes in ethanol at different percentage (2 washes of 7 min each at 20%, 30%, 50%, 75%, 90% and 100%) were done. All washes were made at 4 ℃ and under stirring. The prepared samples were stored in absolute ethanol (99.9%) and subsequently using liquid CO_2 the supercritical dehydration of the samples was conducted.

Afterwards, the samples were placed on an aluminum stub (26 mm) and coated with gold in real time for 100 s using an ionic sputter (E-1010; Hitachi, Tokyo, Japan). Images analyses of coated samples were acquired by using a SEM (Philips 515; Philips, Amsterdam, The Netherlands), with an acceleration voltage of 20.0 kV.

ATR-FTIR spectroscopy

The spectra were acquired using a TENSOR 27 FTIR Bruker spectrophotometer (Bruker, Ettlingen, Germany), equipped with an ATR module provided with a micro-diamond crystal (Specac Quest ATR, Specac Ltd., Orpington, Kent, UK). Each spectrum was the result of an average of 32 scans from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Background spectra were also taken against air under the same conditions before each sample. In addition, all spectra were preprocessed with Grams/386 software (version 6.00, Galactic Industries Corporation, Salem, NH, USA) for baseline correction and subsequently normalized to set the baseline and shift the intensities of the spectra so that the minimum absorbance value was 0.

Raman and SERS spectroscopy

For normal Raman analyses, small fragments (3×3 mm) of treated and untreated LDPE films were washed with absolute ethanol and vortexed in order to remove the majority of the fungal mycelium present on the surface of the treated LDPE film and any other impurity present on the untreated film. After washing and drying, the samples were placed on a microscope slide glass and subsequently analyzed.

For SERS analyses, hydroxylamine silver NPs were prepared as described by Leopold and Lendl [69] by adding 10 mL of silver nitrate solution (10^{-2} M) to 90 mL of a hydroxylamine hydrochloride solution (1.67×10^{-3} M) containing 3.33×10^{-3} M sodium hydroxide. SERS samples were prepared by adding 5 µL of a concentrated silver NPs suspension (10% of the original volume) on the surface of the LDPE-washed films before analyses as previously reported by Puliga et al. [70].

All samples were analyzed under a 50×objective long focal lens. The SERS spectra were obtained by using the portable Renishaw Raman Virsa™ spectrophotometer (Renishaw, Wotton-under-Edge, UK). The excitation laser at 532 nm was employed in all cases, and the laser power was set at 0.5 mW on the samples. All the spectra were obtained with an integration time of 10 s. The spectra were acquired using the Renishaw WiRE 5.5 program and processed with the SpectraGryph software. For each sample, three replicates were made.

Results

Fungal identification and plastic degradation assays

A total of 47 isolates divided into 10 genera belonging to Ascomycota and Mucoromycota were obtained from

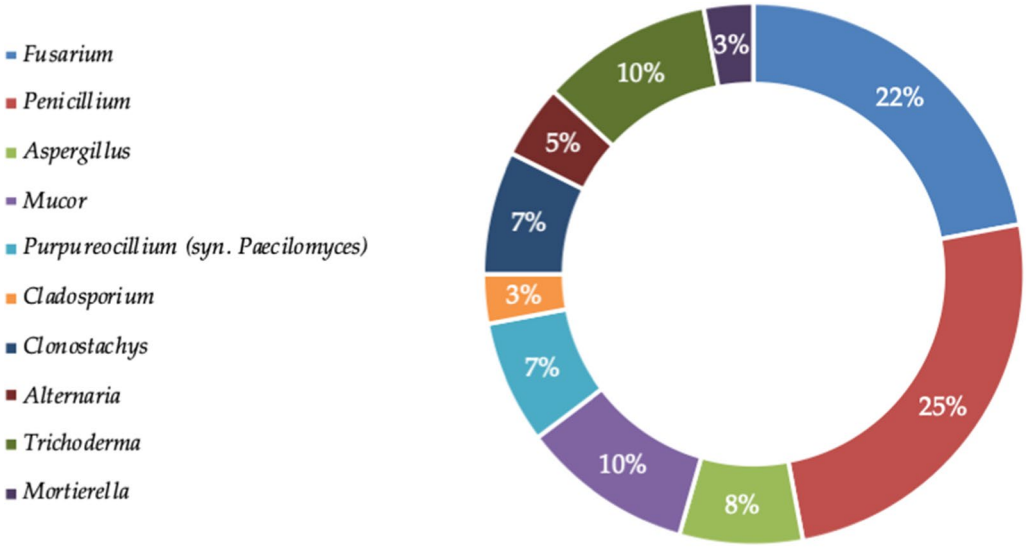


Fig.1 Fungal genera identified in this work

Table 1 Species and their accession number molecularly identified

Species	Isolate (CMI-Unibo)	GenBank accession n°
Cladosporium cladosporioides (Fresen.) G.A. de Vries	Clc/1	OP729904
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	Clr/1	OP729905
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	Clr/2	OP729906
Fusarium sp.	Fus/1	OP729903
Fusarium equiseti (Corda) Sacc.	Fue/1	OP729900
Fusarium equiseti (Corda) Sacc.	Fue/2	OP729907
Fusarium oxysporum Schltdl.	Fuo/1	OP729901
Fusarium sp.	Fus/2	OP729910
Linnemannia elongata (Linnem.) Vandepol & Bonito	Moe/1	OP729902
Mucor fragilis Bainier	Muf/1	OP729908
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson	Pul/1	OP729909

the field-collected plastic debris (Fig. 1). Of these, only 11 isolates were capable of growing on the LDPE substrate and therefore selected for further analyses. Molecular analyses showed that these isolates belonged to 9 species as reported in Table 1. These isolates were deposited in the CMI-Unibo culture collection at the University of Bologna and their ITS sequences were deposited in GenBank (Table 1).

SEM and ATR-FTIR analyses

SEM observations were carried out on the 11 fungal isolates capable of growing on the LDPE film. All tested fungi were capable to adhere to the LDPE film but only *C. cladosporioides* (Clc/1) isolate was able to carry out an early degradation. SEM images of the LDPE film before and after Clc/1 growth are shown in Fig. 2. In particular, small cavities and depressed areas of circular shape are visible in the sample treated with the Clc/1 (Fig. 2b, c) with respect to the untreated LDPE film which is characterized by a smooth surface (Fig. 2a).

ATR-FTIR spectra shown in Fig. 3 were recorded on samples treated with Clc/1, the only isolate that produced clear evidence of plastic surface degradation after SEM observations.

The characteristic absorption bands of the main functional groups were assigned on the basis of those reported by other authors [71–74].

Untreated LDPE film spectrum (Fig. 3, black line) showed two strong bands at around 2915 cm^{-1} and 2852 cm^{-1} due to the -CH_2 asymmetric and symmetric stretching vibrations, respectively. The bands at 1471 cm^{-1} and 714 cm^{-1} corresponded to the – CH_2 scissoring and rocking motions, respectively. Finally, the peak at around 1380 cm^{-1} is used to distinguish LDPE from HDPE [71].

In the spectrum of the treated LDPE (Fig. 3, red line), a significant decrease in the relative intensity of the bands at 2915, 2845, 1471, and 720 cm^{-1} can be seen, indicating an initial degradation of the methylene groups. The appearance of other bands in the spectrum, particularly that at 3263 cm^{-1}, corresponding to the stretching

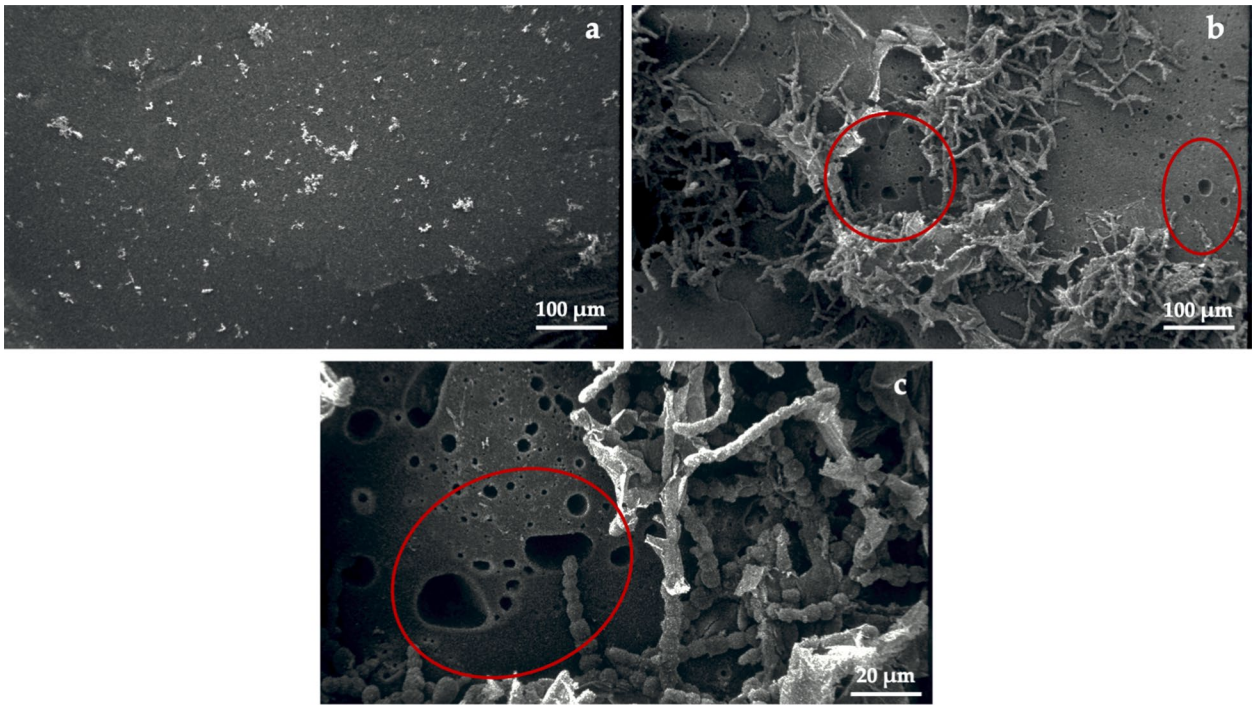


Fig. 2 SEM images of the LDPE film: **a** untreated and **b**, **c** treated with *C. cladosporioides* Clc/1 after 90 days mycelia growth

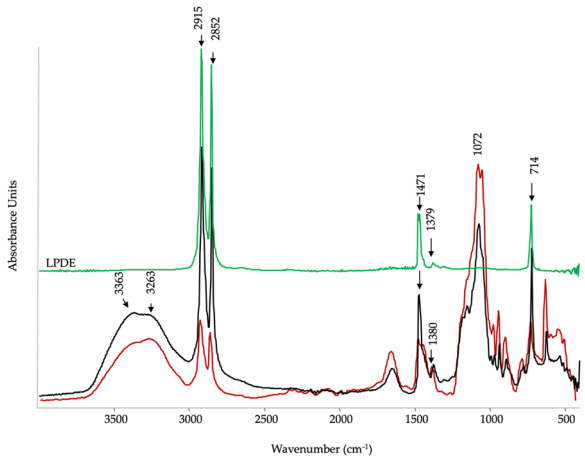


Fig. 3 ATR-FTIR spectra of LPDE film (green line), LPDE treated (red line) and untreated (black line) with *C. cladosporioides* Clc/1 after 90 days of fungal growth

of − OH groups, as well as the one at 1644 cm^{-1} due to amide I and H_2O. Furthermore, the region between 1100 and 1030 cm^{-1} assigned to C–O–C, C–C, C–OH stretching groups of the pyranose ring might be related to the mycelium adhering to the LDPE film surface.

The Raman spectrum of samples treated with Clc/1 together with the untreated LDPE plastic sample is shown in Fig. 4a. These spectra are the average of different spectra registered on a surface of 20 × 20 microns.

Characteristic bands corresponding to the untreated LDPE film spectrum (Fig. 4a, black line) appear at 2878 cm^{-1} and 2844 cm^{-1} attributed to the -CH_2 asymmetric and symmetric stretching vibrations [$\nu_{as}(CH_2)$ and $\nu_s(CH_2)$], respectively. In addition, the intense band at 1430 cm^{-1} is assigned to the − CH_2 scissoring [$\delta_s(CH_2)$], while that at 1286 cm^{-1} is associated with twisting CH_2 vibrations [$\tau(CH_2)$]. Finally, the bands at 1124 and 1054 cm^{-1} are mainly attributed to C–C stretching vibrations [ν(C–C)], and are associated with ordered and disordered polymers chains, respectively [75]. The broad band appearing at 3395 cm^{-1} may indicate the presence of –OH groups derived from a possible spontaneous oxidation of PE chains.

The Raman spectrum of the treated sample (Fig. 4a, red line) displays a similar spectrum but with a slight change in the relative intensity of bands. The difference spectrum (untreated spectrum minus treated one) shown in Fig. 4a (blue line) reveals increasing intensities for the $\nu_s(CH_2)$ at 2878 and the ν(C–C) at 1124 cm^{-1} in favor of the untreated sample. Moreover, the positive band at 1415 cm^{-1}, attributed to crystalline PE [76], also suggests the existence of a more ordered structure in the untreated sample. The relative intensification of these two bands can be associated with a higher structural order in the untreated sample [77, 78].

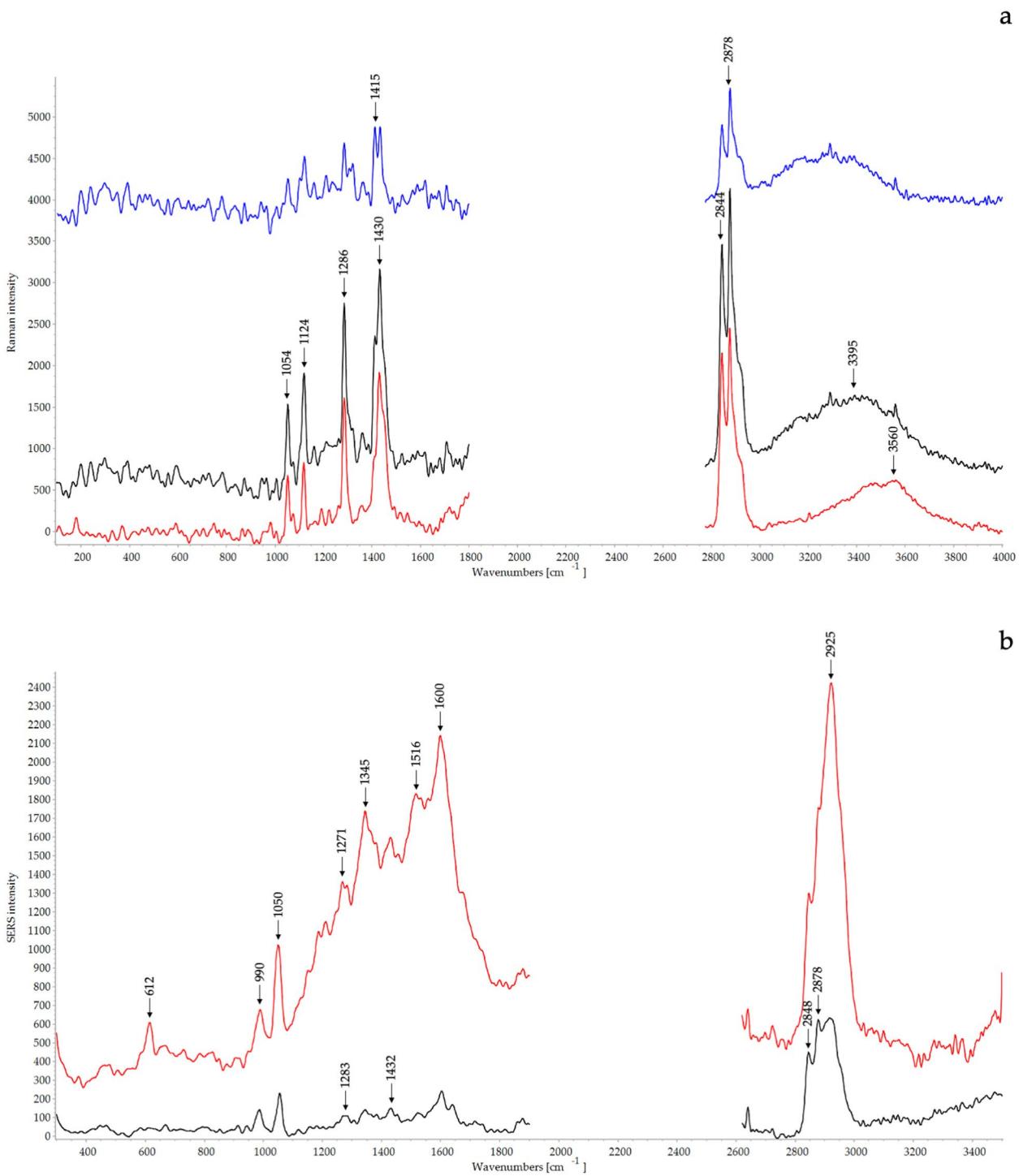


Fig. 4 **a** Normal Raman with the difference spectrum (blue line) and **b** SERS spectra of the untreated LDPE film (control, black line) and treated LDPE film (red line) with *C. cladosporioides* Clc/1 after 90 days of fungal growth

Since the normal Raman spectra only shows slight changes in the LDPE sample upon the action of Clc/1, we have analyzed the same samples by SERS spectroscopy. Figure 4b shows the SERS spectra of the untreated (black line) and treated sample (red line).

The SERS spectrum of the untreated sample reveals interesting changes in relation with the normal spectrum

(Fig. 4b). Although some weak features attributed to the LDPE can be still seen (1283, 1432, 2848 and 2878 cm^{-1}), other bands appear at 990, 1050, 1602, 1632 and 2925 cm^{-1}.

It is noticeable that the SERS effect enhances the oxidized species and other admixtures existing in LDPE. The concentration of these compounds is too low to be actually seen in normal Raman or ATR-FTIR; conversely, as a result of the SERS effect, their intensity is raised. Specifically, these bands reveal the presence of oxygenated compounds that include polyphenols, quinones, and other ones.

The SERS spectrum of the treated LDPE displayed a further enhancement of the bands. In particular, the intense band at 1345 and 1602 cm^{-1} are associated with polyphenol compounds, while the intense band at 2925 can be attributed to $–OCH_3$ moieties.

Discussion

In this work, the isolation of different fungi from plastic residues collected in the fields and their ability to grow and colonize the LDPE were explored. Recently, several studies have been carried out highlighting the ability of different microorganisms to degrade some plastic polymers [26, 27]. Among these fungi are ubiquitous organisms that play a fundamental role in the decomposition of organic matter.

The structural modifications of the LDPE, as a consequence of their growth, were monitored by using conventional techniques such as SEM, ATR-FTIR and normal Raman as well as with an unconventional one which is SERS.

Isolation trials allow to obtain 47 isolates belonging to 10 genera, among which the most abundant were *Penicillium* (25%) and *Fusarium* (22%) followed by *Mucor* (10%) and *Trichoderma* (10%). These results are in line with those previously obtained by Spina et al. [79], where a small number of genera were found associated with plastic residues.

As reported by Lear et al. [26], the presence of PE in the soil leads to a shaping of a peculiar microbiome. In plastic-polluted soils, this implies a reduction in the fungal community with a dominance of a small number of species, mainly ascomycetes, as already observed by Raghavendra et al. [32].

Among all the 47 fungal isolates, 11 were able to grow and colonize the LDPE film, but only Clc/1 seems to be able to carry out an initial degradation of the LDPE substrate. Brunner et al. [38] showed that a strain of this species, isolated from plastic debris floating in the shoreline of a lake, was able to degrade polyurethane but not PE. Since our results are in contrast with those reported by Brunner and collaborators [38], we could hypothesize that the ability of *C. cladosporioides* to degrade LDPE is strain specific.

The degradative signs observed with the SEM are similar to those reported in the literature by Austin et al. [80]. Thanks to the production of non-specific enzymes, fungi are able to attack complex and recalcitrant xenobiotics such as plastics [51, 54]. Laccases, peroxidases, lipases, esterases and cutinases enzymes are involved in the depolymerization of the long carbon chains of plastic polymer into simpler and more degradable oligomers, dimers, and monomers [81]. Regarding the PE degradation, the main fungal enzymes involved in the degradation seem to be the ligninolytic laccases and peroxidases [82]. The ability of *C. cladosporioides* to produce laccases and peroxidase was previously investigated by other authors [83–86].

ATR-FTIR analysis carried out after 90 days of fungal growth supports the observed structural changes. The most significant modifications were observed in the methylene chains, as already found by other authors [39, 43, 79]. The decrease in the relative amount of these functional groups suggests that Clc/1 is capable of breaking the C–H bonds of the polymer chain.

Normal Raman spectra, in agreement with FT-IR, showed the alkyl chain modifications and, more specifically, exhibited a partial disorder of the polymer structure as a result of Clc/1 activity, probably due to the enzymatic action on the alkylic chains. SERS spectroscopy displayed more sensitivity in the analysis by detecting oxygenated compounds, including polyphenols, quinones and admixtures that are also present in the LDPE structure.

Conclusions

Plastic pollution is becoming one of the biggest and global problems which is far to be solved. Biodegradation of plastic polymers by microorganisms has been considered one of the most promising approaches for solving plastic pollution. The application of SEM, ATR-FTIR, normal Raman and SERS spectroscopies has shown different detection level of an early LDPE film degradation process by Clc/1 strain. Our study highlighted that normal Raman combined with SERS are promising spectroscopic techniques for a rapid screening and selection of the microorganisms for plastic biodegradation in the environment. The early plastic degradation of the isolated strain of *C. cladosporioides* (Clc/1) showed that it can contribute to plastic degradation if introduced in natural environment. Further study involving genome sequencing and transcriptome analyses of this strain will be necessary to better understand the Clc/1 enzymatic pathways. In particular, the identification of the enzymatic

pattern responsible for plastic degradation can lead to their biosynthesis for future biotechnological application.

Abbreviations

SERS	Surface-enhanced Raman scattering
FT-IR	Fourier transform infrared
NPs	Nanoparticles
LDPE	Low density polyethylene
SEM	Scanning electron microscope
ATR-FTIR	Attenuated total reflectance-Fourier transform infrared
PDA	Potato dextrose agar
ITS	Internal transcribed spacer
PCR	Polymerase chain reaction

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Author contributions
Conceptualization, FP; methodology, FP, VZ, DB, DC and SSC; formal analysis, FP, VZ and SSC; investigation, FP, VZ, DC and DB; resources, AZ, OF and SSC; data curation, FP and VZ; writing—original draft preparation, FP; writing—review and editing, FP, VZ, AZ, OF and SSC; visualization, FP and DB; supervision, AZ, OF and SSC; funding acquisition, AZ, OF and SSC. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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