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Microbial consortia for multi-plastic waste biodegradation: Selection and validation

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ABSTRACT

The accumulation of plastic waste in the environment poses significant ecological and health risks. This study evaluates the effectiveness of microbial consortia in degrading various types of plastics, including low-density polyethylene (LDPE), low linear-density polyethylene (LLDPE), polyethylene terephthalate (PET) and polystyrene (PS). Qualitative enzyme assays for esterases and ligninases, which are related to plastic biodegradation, were conducted in five microbial strains. Four microbial consortia, combining bacterial and fungal strains, were assembled based on the enzyme profile of their components and evaluated for their ability to degrade both virgin and recycled plastics. The results showed that consortia C2 (*Bacillus subtilis* RBM2, *Fusarium oxysporum* RHM1, and *Alternaria alternata* RHM4) and C4 (*Bacillus subtilis* RBM2 and *Pseudomonas alloputida* REBP7) exhibited the highest biodegradation efficiency, particularly achieving significant weight loss in recycled LDPE, virgin LLDPE and recycled PET. The biodegradation was further confirmed by FTIR analysis, which revealed changes in the chemical composition and functional groups of the treated plastics, indicating microbial interaction and degradation. This study underscores the potential of microbial consortia in addressing plastic pollution, highlighting the importance of strategic consortia design based on enzymatic profiles and plastic colonization capabilities. These promising results suggest that further optimization of microbial consortia could offer a viable solution for large-scale plastic waste management.

1. Introduction

Global plastic production is increasing at an alarming rate due to human population growth, rapid urbanization, and industrialization. The unique properties of plastics, i.e. their light weight, durability, mechanical strength, and chemical and corrosion resistance, make them versatile material for a wide range of applications (Vasilopoulou et al., 2021). In 2022, 400.3 million tons of plastic were produced worldwide, which is expected to quadruple by 2050 (PlasticsEurope, 2023). This high plastic production results in the

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generation of large amounts of plastic waste. Zhu et al. (2023) reported that 12,000 tons of plastic waste will be transported from land to the ocean via rivers or coastlines in the next years. These practices are not aligned with the concept of sustainable development, as the breakdown of plastics due to physicochemical factors leads to the formation of microplastics and nanoplastics, which can have harmful impacts on human health and ecosystems (Mong et al., 2023). This contributes to serious environmental impacts leaving a lasting footprint on the planet, as most plastics are primarily composed of petrochemical hydrocarbons with additives such as stabilizers, oxidizers and flame retardants that hinder their biodegradation (Su et al., 2022). Thus, plastic production has led to significant plastic waste accumulation to the extent that this situation characterizes the current era, known as the Anthropocene or the plastic age (Mong et al., 2023; Porta, 2021).

Most plastics used in industry and agriculture are composed of polyethylene terephthalate (PET), polyethylene (PE) and polystyrene (PS) (Du et al., 2021). The disposal of these plastics is one of the most significant challenges in plastic waste management due to the expensive and time-consuming technology currently available (Jacobs et al., 2022). The primary drawback in addressing this challenge is their resistance to biodegradability, mainly due to their stable structure and the lack of eco-friendly recycling solutions. Despite ongoing research and the development of various pre-treatment techniques, no widespread solution for recycling multilayered plastics (composed of two or more plastic polymers) is expected in the next 5–10 years (Mello Soares et al., 2022). In this context, using biological tools for plastic degradation is increasingly recognized as a sustainable approach to address the accumulation of plastic waste in the environment (Priya et al., 2022).

Microorganisms play a key role in the removal of recalcitrant compounds such as hydrocarbons or phenolics from the environment, due to the wide range of metabolic activities they exhibit. Therefore, the search for microorganisms with the necessary metabolic capabilities to degrade plastics has been proposed as a promising approach to plastic waste management. Several microbial enzymes have been reported to be closely related to the metabolism of plastics. Lignin-degrading enzymes, such as laccases and polyphenol oxidases, could destabilize the highly crystalline structure of plastics that lack reactive groups, like polyethylene (Dey et al., 2020). Esterases, such as lipases and cutinases, are well-studied for their role in plastic biodegradation, where they cleave ester bonds in polymer plastics such as PET and polyurethane (PU) (Temporiti et al., 2022). Different enzymes can be simultaneously involved, for example, *Rhodococcus* species have demonstrated the ability to degrade PE through the action of a lipase and a multicopper oxidase (Tao et al., 2023). The biodegradation of LDPE by *Bacillus cereus* has been linked to the production of laccases and lipases (Jayan et al., 2023). These enzymes catalyze oxidative and hydrolytic processes that can break down the polymer chains of polymers such as PE, which is typically resistant to degradation due to its high crystallinity and lack of reactive functional groups. Moreover, the ability of microorganisms to colonize plastics by forming biofilms plays a key role in biodeterioration and further breakdown of plastics polymers (Zhai et al., 2023).

Since individual microbial strains often lack the complete set of enzymes required for efficient plastic degradation, using diverse microorganisms with complementary enzymatic activities is proposed as a promising solution for plastic waste treatment. This association of microorganisms is known as a microbial consortium. These consortia cover a wider range of enzymatic activities, which may lead to greater efficiency in degrading various types of plastics compared to individual strains. Microbial consortia have already been applied for the bioremediation of environments contaminated with other recalcitrant molecules such as phenolic compounds (Martínez-Gallardo et al., 2021). Additionally, the efficiency of plastic biodegradation is influenced by the format of the plastic. Virgin polymers are generally less susceptible to microbial degradation than recycled plastics, whose molecular structure may be altered due to the recycling process (Tokiwa et al., 2009).

An efficient and sustainable strategy for the biodegradation of highly recalcitrant plastics, such as mixed plastic waste, is essential due to its significant impact on both the environment and human health. Although many studies have explored the use of microbial consortia for the biodegradation of conventional plastics, most have focused on one or two specific polymers (Syranidou et al., 2019; Skariyachan et al., 2018, 2021; DSouza et al., 2021). However, flexible microbial consortia capable of degrading a wide range of plastic polymers have yet to be developed.

The main objective of this work is to investigate the effectiveness of microbial consortia as a biotechnological tool for the biodegradation of various virgin and recycled plastics polymers, such as polyethylene terephthalate (PET), polystyrene (PS), low-linear density polyethylene (LLDPE) and low-density polyethylene (LDPE). The novelty of this research lies in assembling microbial consortia based on their enzymatic complementarity and their capacity to degrade a broad spectrum of plastics, both virgin (V) and recycled (R). This approach seeks to simulate natural environmental conditions, enhancing the potential for practical application in real-world scenarios. To achieve this the following goals were established: (1) to select combinations of microorganisms that could be suitable for multi-plastic degradation, based on the physiological profiles related to plastic metabolism, and (2) to demonstrate the effectiveness of selected microbial consortia in degrading virgin and recycled multi-plastics.

2. Material and methods

2.1. Microorganisms

Three bacteria, *Bacillus subtilis* RBM2, *Bacillus altitudinis* RBM10, *Pseudomonas alloputida* REBP7 and two fungi, *Fusarium oxysporum* RHM1, *Alternaria alternata* RHM4, were used for the study. These microorganisms are part of the microbial collection of the BIO175 research group of the University of Almeria (Spain) and were previously isolated from compost (Jurado et al., 2014) or plastic contaminated microcosm (Salinas et al., 2023). The strains were stored in cryovials at −80 °C and reactivated on nutrient agar after incubation at 30 °C for 24 h.

2.2. Plastic-degrading activities

Enzymatic activities related to plastic degradation were tested in the strains under study, including: esterases, such as lipase (LIP) (EC 3.1.1.3) and cutinases (CUT) (EC 3.1.1.74), and ligninases (LIG), such as laccases (LAC) (EC 1.10.3.2) and polyphenol oxidase (PO) (EC 1.10.3.1). Pure cultures on Potato Dextrose Agar (PDA, Panreac) and Nutrient Agar (NA, Panreac) media, for fungi and bacteria, respectively, were inoculated in specific agar culture media to study the presence of the enzymes mentioned. For the detection of LIP, CUT1 and CUT2, media with tributyrin, glycerol monoesterate and polycaprolactone were used, respectively (Molitor et al., 2020). LIG enzymes were demonstrated by decolorizing RBBR (Remazol Brilliant Blue Dye), PO was detected by observing brown colored halos in an acid tannic-containing medium (Martinez-Gallardo et al., 2020). LAC were revealed by adding the reagents α-naphthol (LAC1) and guaiacol (LAC2) to colonies growing on NA plates (López et al., 2006; Martínez-Gallardo et al., 2020).

The colonization of plastic was evaluated on NA Petri dish in which LLDPE film was placed and microorganisms were inoculated. First, 1 cm^2 LLDPE films were sterilized by soaking in alcohol for 45 min and then dried at 40 ℃ under sterile conditions. Subsequently, LLDPE films were placed on the agar surface. For bacterial inoculation, 10 μL of a 48 h pre-inoculum incubated at 30 °C in nutrient broth (Panreac, Spain) was placed on the LLDPE films. For fungi, a 0.5 cm diameter plug of 5 days-old fungal inoculum on PDA was placed on the plastic film. After 3 and 15 days incubation at 30 °C for bacteria and fungi, respectively, microbial growth on the plastic was qualitatively evaluated by adding 10 μL of 0.01 % (w/v) aqueous resazurin solution to the inoculated areas. The plates were then incubated for 2 h in the dark at 30 °C. A positive result, indicating the ability to grow on the plastic surface, was identified by the reduction of resazurin (blue) to resorufin (pink) (Chadha and Kale, 2015).

2.3. Establishment of the plastics degradation assay

To evaluate the effectiveness of each microbial consortium for plastic degradation, a study was conducted using plastic as the sole carbon source for the growth of the consortia microorganisms. For this purpose, microplastics (particle size < 2 mm) were sterilized by immersing them in 70 % v/v ethanol for 45 min, followed by complete drying at 40 ℃ under sterile conditions. The experiment was carried out in 100 mL flasks, each containing 50 mL of M9 minimal salt medium supplemented with 1 % (w/v) of LLDPE, LDPE, PET, or PS, in virgin (V) and recycled (R) forms. The medium was inoculated with a 1 % (v/v) suspension of 10^6 CFU/mL (bacteria) or 10^5 CFU/mL (fungi) of each member of the consortium. The flasks were incubated at 30 °C and 120 rpm for 30 days. The liquid phase was collected at the end of the incubation period for quantifying viable cells while the microplastics were subjected to gravimetric analysis both before and after the incubation period to assess plastic degradation. Microbial growth was analyzed by colony counts on NA medium for bacteria and Rose Bengal (Panreac) for fungi, and the results were expressed as colonies forming units per milliliter (CFU/mL).

2.4. Analysis of plastic biodegradation by weight loss

The plastic was recovered by filtering the culture medium, and the microplastic remaining on the filter was washed with 2 % (w/v) sodium dodecyl sulfate (SDS) solution for 4 h to detach microbial biomass. Subsequently, the SDS solution was removed, and the plastic was washed with distilled water until no residue remained on the filter and dried for 24 h at 40 ℃. Weight was measured using a microbalance (Sartorius Biotech, Germany). The percentage of weight loss was determined using the following equation, as given in Skariyachan et al. (2021):

$$\text{Weight loss (\%)} = ((W_0 - W_f) / W_0) \times 100$$

Where, $\mathbf{W_0}$ is the initial weight included in minimal media and $\mathbf{W_f}$ is the final weight after the incubation period.

2.5. Fourier transform infrared analysis

FTIR technique has been used to obtain information on the functional groups that characterise the polymeric structure of the material allowing its identification. Infrared spectroscopic analysis was performed using an FTIR ATR Spectrum 400 Perkin-Elmer (germanium crystal) spectral sensitivity 4 cm^{-1}, in the frequency range of 4000–650 cm^{-1}. Morphological and FTIR analysis of sample surfaces was also performed using the optical microscope combined with a FTIR Spotlight Chemical Imaging Perkin Elmer instrument (300X magnification).

2.6. Statistical analysis

An analysis of variance (ANOVA; $p < 0.05$) was conducted for all variables across the experiments. Fisher's Least Significant Difference (LSD) test was used to compare the mean values of different treatments and assess whether there were any significant differences among them. The results of the statistical analysis are presented in figures with error bars representing the LSD interval. Bars with different letters were found to be significantly different based on Fisher's LSD test ($p < 0.05$). All experimental conditions and analyses were performed in triplicate, and data are presented as the mean. Data normality and homogeneity were confirmed using the Shapiro-Wilk test. Statistical analyses were carried out using Statgraphics Centurion XIX version 19.4.01 (Stat-Point, Inc.).

Table 1
Microbial composition of each consortium selected for plastic degradation based on the expression of different enzymes such as Lipase (LIP), Cutinase 1 (CUT1), Cutinase 2 (CUT2), Laccase 1 (LAC1), Laccase 2 (LAC2), Polyphenoloxidase (PO), and Ligninase (LIG); as well as plastic colonization capacity (COL). The colored cells represent positive results. Each color corresponds to a different consortium.

Consortia	Microoranisms	Group	LIP	CUT 1	CUT 2	LAC 1	LAC 2	PO	LIG	COL
C1	*B. subtilis* RBM2	Bacteria								
	F. oxysporum RHM1	Fungi								
C2	*B. subtilis* RBM2	Bacteria								
	F. oxysporum RHM1	Fungi								
	A. alternata RHM4	Fungi								
C3	*B. subtilis* RBM2	Bacteria								
	B. altitudinis RBM10	Bacteria								
C4	*B. subtilis* RBM2	Bacteria								
	P. alloputida REBP7	Bacteria								

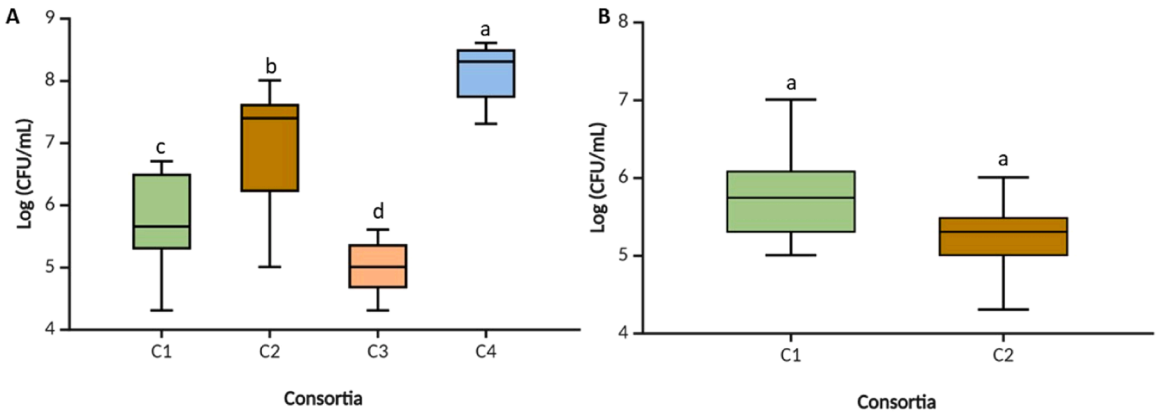

Fig. 1. Boxplot showing growth of bacterial (A) and fungal (B) members of microbial consortia, expressed as the logarithm of colony forming units per milliliter (Log CFU/mL), after 30 days of incubation in liquid medium with plastic as the sole carbon source. Consortium 1 (C1): *B. subtillis* RBM2 and *F. oxysporum* RHM1; Consortium 2 (C2): *B. subtillis* RBM2, *F. oxysporum* RHM1 and *A. alternata* RHM4; Consortium 3 (C3): *B. subtilis* RBM2 and *B. altitudinis* RBM10; Consortium 4 (C4): *B. subtilis* RBM2 and *P. alloputida* REBP7. The values within the box are the average of three replicates. The boxes bearing different letters were significantly different according to Fisher's LSD test ($p < 0.05$).

3. Results and discussion

3.1. Evaluation of microbial collection capacities and consortia assembly for plastic biodegradation

Based on the complementarity of their enzymatic profiles, covering a broad spectrum of plastic biodegradation capacities, the five microbial strains analysed in this study were assembled into four consortia. Table 1 shows these combinations according to the plastic degradation-related activities expressed by each microorganism in each consortium. The efficiency of plastic degradation can be enhanced by forming microbial consortia that leverage the unique enzymatic capabilities of each microorganism. Several reports have confirmed the consistency of the microorganisms selected for the assembly of consortia. The genus *Bacillus*, particularly *B. subtilis* RBM2, exhibited significant enzymatic potential related to plastic degradation. This bacterium had the ability to colonize the LLDPE surface and express a wide range of activities related to plastic degradation, including laccase activity (Jurado et al., 2014). Other reports also demonstrated the capacity of *Bacillus* and related genera to biodegrade polymers like PE (Skariyachan et al., 2018; Jayan et al., 2023). Likewise, the bacterium *P. alloputida* REBP7 displayed an enzymatic profile relevant to plastic metabolism, including lignin-degrading enzymes such as ligninase (LIG) and polyphenol oxidase (PO). *Bacillus* spp. and *Pseudomonas* spp. have been extensively studied by some authors as effective plastic biodegraders when used in consortia (Roberts et al., 2020; Wu et al., 2023; Yan et al., 2023). Their ability to degrade a wide range of recalcitrant compounds, including plastics such as PE, PET or PS, through specific enzymatic activities is demonstrated in several studies (Liang et al., 2022; Yan et al., 2023; Kučić Grgić et al., 2023). Among the fungal strains, *Alternaria alternata* RHM4 exhibited the widest range of enzymatic activities related to plastic degradation. These results agree with those obtained by Gao et al. (2022), who found a strain of *A. alternata* FB1 that possesses 153 enzymes potentially involved in PE biodegradation, as revealed by transcriptomic analysis. The fungus *Fusarium oxysporum* RHM1 expressed two key enzymes, LIP and CUT1, both of which (lipases and ligninases) play crucial roles in plastic metabolism (Temporiti et al., 2022). To create efficient

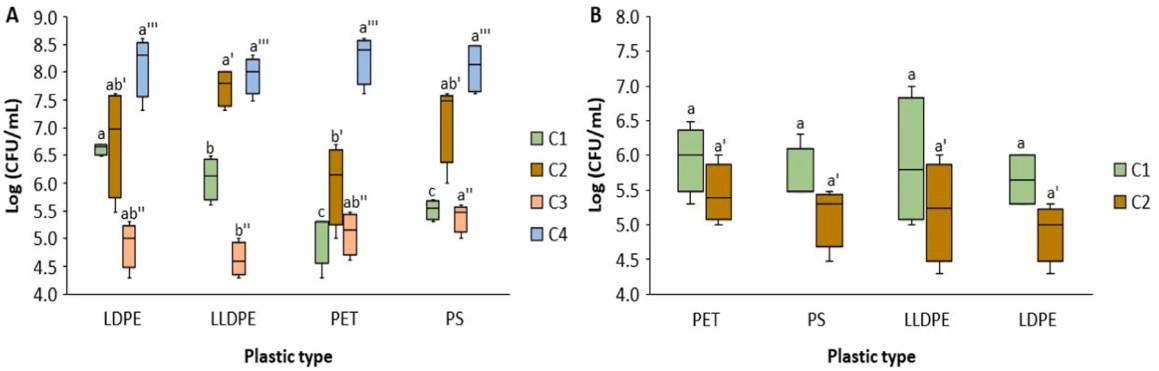

Fig. 2. Boxplot showing growth of bacterial (A) and fungal (B) members of the microbial consortia, expressed as the logarithm of colony forming units per milliliter (Log CFU/mL), after 30 days incubation in liquid media with LDPE, LLDPE, PET and PS, as the sole carbon source. Consortium 1 (C1): *B. subtillis* RBM2 and *F. oxysporum* RHM1; Consortium 2 (C2): *B. subtillis* RBM2, *F. oxysporum* RHM1 and *A. alternata* RHM4; Consortium 3 (C3): *B. subtilis* RBM2 and *B. altitudinis* RBM10; Consortium 4 (C4): *B. subtilis* RBM2 and *P. alloputida* REBP7. The values within the box are the average of three replicates. The boxes bearing different letters were significantly different according to Fisher's LSD test ($p < 0.05$).Plastic biodegradation by weight loss.

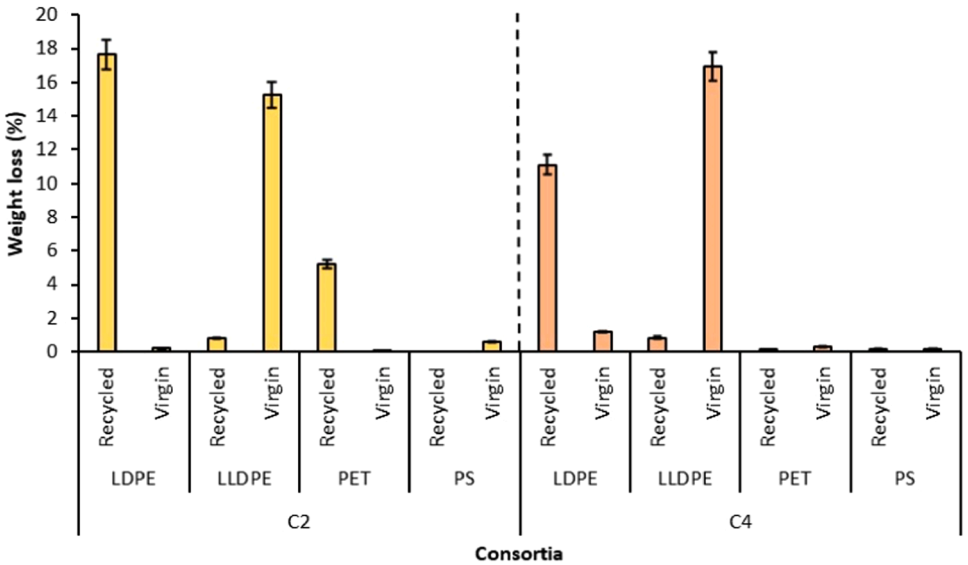

Fig. 3. Weight loss, expressed in percentage (%), of virgin and recycled LDPE, LLDPE, PET and PS tested after exposure to the C2 and C4 consortia for 30 days in liquid media. Consortium 1 (C1): *B. subtillis* RBM2 and *F. oxysporum* RHM1; Consortium 2 (C2): *B. subtillis* RBM2, *F. oxysporum* RHM1 and *A. alternata* RHM4; Consortium 3 (C3): *B. subtilis* RBM2 and *B. altitudinis* RBM10; Consortium 4 (C4): *B. subtilis* RBM2 and *P. alloputida* REBP7. Results are means (n = 3) ± SD (vertical bars). Error bars represent Fisher's LSD interval ($p < 0.05$).

microbial consortia for plastic degradation, it is essential to consider both the enzymatic profile of the microorganisms and their ability to colonize plastic surfaces. Since *B. subtilis* RBM2 demonstrated the most promising enzymatic activity for plastic biodegradation, it was a crucial component in the consortia, which were composed of bacterial and fungal species in pairs or trios. Based on these criteria, the proposed consortia were as follows: C1, composed of *B. subtillis* RBM2 and *F. oxysporum* RHM1; C2, composed of *B. subtillis* RBM2, *F. oxysporum* RHM1 and *A. alternata* RHM4; C3, consisting of *B. subtilis* RBM2 and *B. altitudinis* RBM10; and C4, consisting of *B. subtilis* RBM2 and *P. alloputida* REBP7 (Table 1).

3.2. Selection of multi-plastic degrading consortia

To evaluate the potential range of plastic degradation, the capability of the consortia to grow on culture medium with virgin or recycled PET, PS, LDPE and LLDPE as the sole carbon source was evaluated. Fig. 1 shows the overall growth of the bacterial and fungal (if any) components of the consortia, regardless of the tested plastic, providing information on the abilities of the microbial consortia to grow using plastic as a substrate. In general terms, bacterial members demonstrated the ability to proliferate from plastic as the sole

substrate, exceeding the initial inoculum loading values (10^6 and 10^5 CFU/mL, for bacteria and fungi respectively) during the incubation period (Fig. 1A). Among all the plastic polymers, the highest growth rates of bacterial components were observed in *B. subtilis* RBM2 on C2 (9–7 Log CFU/mL) and *B. subtilis* RBM2 + *P. alloputida* REBP7 on C4 (8–5 Log CFU/mL). These two consortia exhibited a substantial increase in cell numbers by 3 and 4 Log units, respectively, after 30 days incubation. As for the fungal members in the two consortia containing fungi (C1 and C2), both were able to grow from plastics increasing inoculum levels by approximately 2–1 Log CFU/mL from the starting time. The growth levels were quite high considering the restrictive nutritional conditions (Skariyachan et al., 2018; Roberts et al., 2020).

Fig. 2 shows the cell levels of bacterial (A) and fungal (B) members of the different consortia after 30 days incubation on culture media containing virgin and recycled PET, PS, LDPE and LLDPE as the sole carbon source. Bacterial members demonstrated the ability to grow and, in most cases, exceed the initial cell load by the end of the incubation period (Fig. 2A). In particular, *B. subtilis* in C4 reached high growth levels on all plastic types, showing a significant increase in cell concentration compared to the initial load, reaching the highest levels among all the bacteria tested. *B. subtilis* in C2 followed a similar pattern, showing the highest growth in the presence of LLDPE (9–7 Log CFU/mL). Bacteria in the other consortia exhibited either a slight increase in cell concentration on the different plastics or maintained their initial levels. Regarding the fungal members, *F. oxysporum* RHM1 in C1 was able to grow and surpass its initial load on all plastic types. In particular, in the presence of PET, the fungus showed a slightly higher performance than the other plastics (Fig. 2B). This fungus is reported to be very versatile for plastic degradation, including LDPE and PET (Spina et al., 2021; Cognigni et al., 2023).

Based on the ability of C2 and C4 to proliferate in the presence of a wide range of plastics as a sole carbon source, subsequent analyses focused on these consortia with the aim of reaching a more complete understanding of their ability to interact with these plastics.

The C2 and C4 consortia were selected based on their ability to grow in culture media with the different plastics studied as the sole carbon source. The efficiency of each consortium to degrade the plastics was evaluated by measuring the weight loss of the PET, PS, LDPE and LLDPE polymers, both virgin and recycled, after 30 days of incubation (Fig. 3). In general, all plastics were degraded to varying extents by both consortia, except for recycled PS inoculated with C2. The highest weight losses, above 10 %, were observed in the different types of polyethylene, particularly in recycled LDPE and virgin LLDPE. These remarkable results may be attributed to the ligninolytic mechanisms expressed by members of both consortia, as well as the possible production of biosurfactants associated with *Bacillus* genus, as reported by Mukherjee et al. (2018). Specifically, C2 led to a higher weight loss in recycled LDPE (17.65 %) compared to virgin LLDPE (15.27 %), while the C4 consortium caused weight reductions of 11.11 % and 16.94 % in recycled LDPE and virgin LLDPE, respectively (Fig. 3). PET was significantly degraded only by C2, and exclusively in its recycled form, with a weight loss around 6 %. In the case of PS, no degradation was obtained under any condition (Fig. 3).

Considering the limited incubation period of 30 days, and the high recalcitrance of the plastics under this study, the biodegradation levels are particularly remarkable. While higher degradation rates have been reported by other authors (over 60 % weight loss in 14 days), these studies used less recalcitrant polymers, such as PU (Álvarez-Barragán et al., 2016). On the other hand, previous studies using single cultures of *P. aeruginosa* WD4 showed very low biodegradation of LDPE (around 9 % weight loss) even after 100 days incubation (Shilpa et al., 2023). This highlights the advantage of using microbial consortia to enhance the breakdown of highly degradation-resistant polymers. Similar biodegradation rates have been reported in other microbial consortia studies. For instance, *Bacillus* sp. and *Paenibacillus* sp. showed a PE weight reduction of 14.7 % after 60 days of incubation (Park and Kim, 2020), while *Stenotrophomonas* sp. and *Achromobacter* sp. resulted in a LDPE weight loss of approximately 8 % (Dey et al., 2020). However, further improvements in the biodegrading capabilities of these consortia are still possible by emplying strategies such as extended incubation times, higher temperatures, or polymer pretreatment by UV radiation (He et al., 2023). For instance, a consortium of *Aspergillus niger*, *A. flavus*, and *A. oryzae* achieved a 26.15 % reduction in LDPE weight after 55 days (DSouza et al., 2020), and co-cultures of *Enterobacter* sp. and *Pseudomonas* sp. resulted in a 64 % degradation of UV pre-treated LDPE after 160 days incubation (Skariyachan et al., 2021). Additionally, biodegradation rates higher than 60 % have been reported using consortia incubated for 120–140 days at temperatures above 50 °C (Skariyachan et al., 2017, 2018).

In general, the promising results observed in the multi-plastics biodegradation assay with C2 and C4 consortia could be attributed to the expression of laccase activity by *Bacillus subtilis* RBM2 and its notable ability to colonize the plastic. Additionally, this colonization capacity seems to be supported by the presence of the other microorganisms in the consortia in both cases (Cao et al., 2022). Furthermore, the enhanced plastic degradation efficiencies noted in consortia C2 and C4 can be significantly linked to the action of lignin-degrading enzymes, particularly laccases and lignin peroxidases (PO). Recent studies have shown that ligninase enzymes can effectively degrade complex aromatic compounds found in non-biodegradable plastics, thereby facilitating their breakdown (Bautista-Zamudio et al., 2023; Temporiti et al., 2022). Given the promising results of recycled LDPE, virgin LLDPE, and recycled PET plastics obtained from the plastic weight assay, these plastics were subjected to further analytical testing to elucidate some of the degradation mechanisms involved.

3.3. Plastic biodegradation: FTIR, and molecular weight analysis

The following plastic samples treated with the consortia (30 days) were subjected to further morphological, thermal and chemical analysis to determine the impact of consortia treatment on the structure of the plastic by comparison to untreated samples: recycled PET, recycled LDPE, and virgin LLDPE, treated with C2; recycled LDPE and virgin LLDPE, treated with C4.

Figs. 4 and 5 report the Chemical Imaging and FTIR analysis carried out on the surface of the samples of recycled LDPE untreated, treated with C2, and treated with C4.

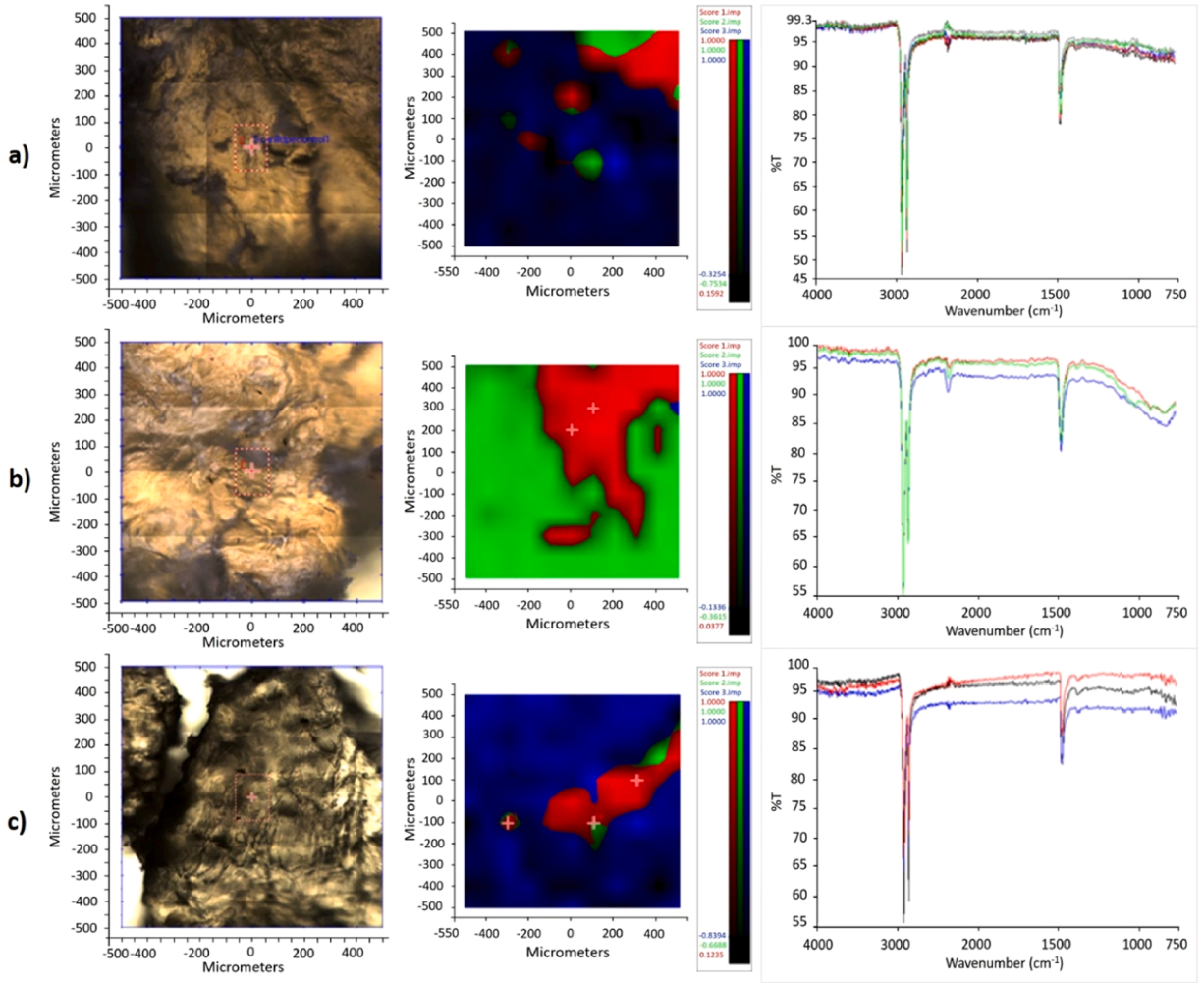

Fig. 4. Chemical Imaging and FTIR analysis results on recycled LDPE samples untreated (a), treated with C2 (b), and treated with C4 (c): optical images (left), chemical maps (middle), and FTIR spectra (right) of the samples' surface areas.

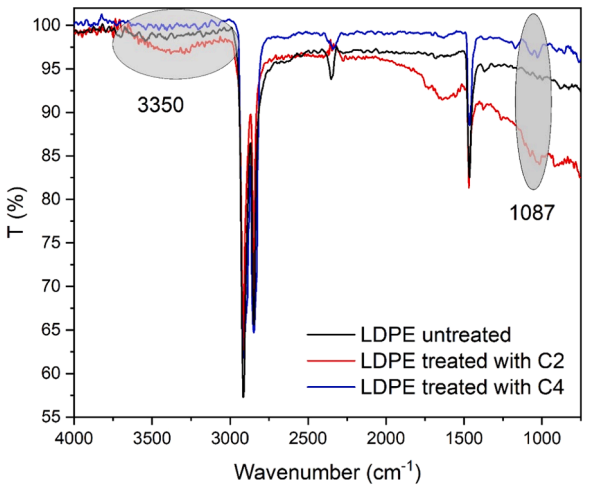

Fig. 5. Comparison of FTIR spectra of recycled LDPE samples untreated (black) treated with C2 (red), and treated with C4 (blue). The main differences are displayed in the grey circles.

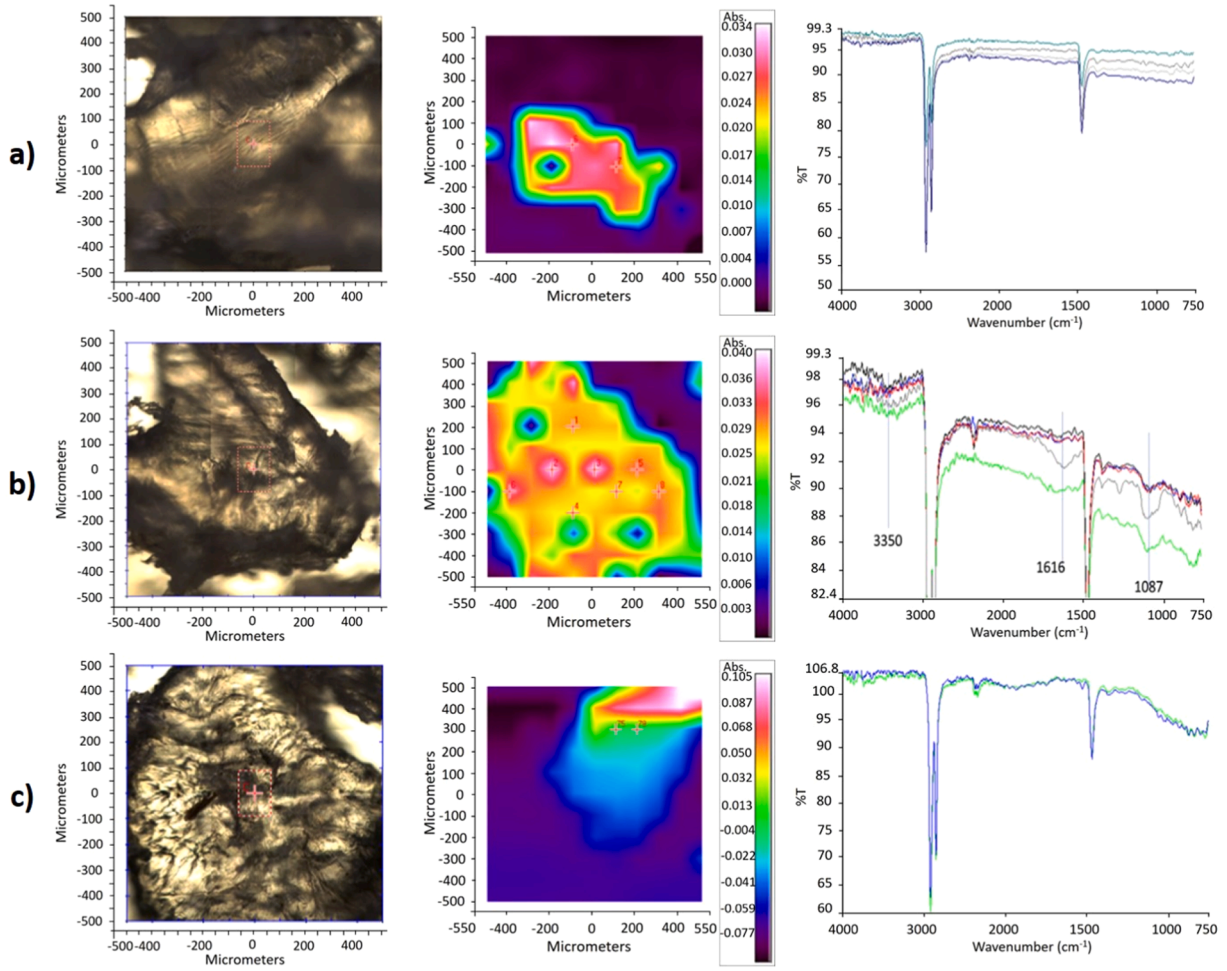

Fig. 6. Chemical Imaging and FTIR analysis results on virgin LLDPE samples untreated (a), treated with C2 (b), and treated with C4 (c): Optical images (left), chemical maps (middle), and FTIR spectra (right) of the samples' surface areas.

The Chemical Imaging and FTIR analysis carried out on the surface of the samples of recycled LDPE untreated, treated with C2, and treated with C4, revealed some changes in the chemical composition of the surface areas ascribed to the treatment with the consortia C2 and C4 (Figs. 4 and 5). As shown in Fig. 5, all the FTIR spectra presented the peaks in the range 2900–2850 cm^{-1} due to the methyl and methylene CH stretching, and the bending vibration of methyl and methylene groups at 1375 and 720 cm^{-1}, typical of polyethylenes. Additional peaks at 1087 cm^{-1} and 3350 cm^{-1} related to C-O and OH groups, respectively, were displayed in the spectra of LDPE samples exposed to microbial consortia C2 and C4, indicating the presence on the samples' surface of functional groups containing oxygen.

Analogous results were obtained by Chemical Imaging and FTIR analysis performed on the virgin LLDPE samples and reported in Fig. 6.

This result is in agreement with what reported in literature for polyethylene samples exposed to larvae and worms (Bombelli et al., 2017). Some authors described the presence of the peak around 3300 cm^{-1} as a signature for ethylene glycol presence attributed to PE degradation. Nevertheless, not all the authors detected the presence of ethylene glycol after the PE degradation (Bombelli et al., 2017). FTIR results of the analysed samples revealed the degradation of LDPE and LLDPE samples, however, further investigations are needed to completely understand the mechanism.

The Chemical Imaging and FTIR analysis results of recycled PET untreated and treated with C2 are reported in Fig. 7. A comparison of FTIR spectra of recycled PET untreated and treated with C2 is also reported in Fig. 8. As can be noted, FTIR spectra of recycled PET showed the characteristic absorption bands of this polymer: the aromatic ring (C-H) at 1408 cm^{-1}, the wagging of the methylene group w(CH2) at 1340 cm^{-1}, the carbonyl stretching ν(C═O), located at 1716 cm^{-1}, the stretching of the ester bond ν(C═O)-O, at 1242 cm^{-1}, the stretching of the glycolic bond ν(O-CH2), at 1095 cm^{-1}, and peaks between 750 cm^{-1} and 1100 cm^{-1} related to C–H bond stretching (methylene group). Compared to the untreated recycled PET, the sample treated with consortia C2 showed differences in the range between 1500 and 1700 cm^{-1}, related to carboxyl or carboxylate or amide I band, and evidence of a band in the range 3100–3500 cm^{-1}, related to the presence of OH functionalities, thus indicating changes in the chemical composition of the surfaces.

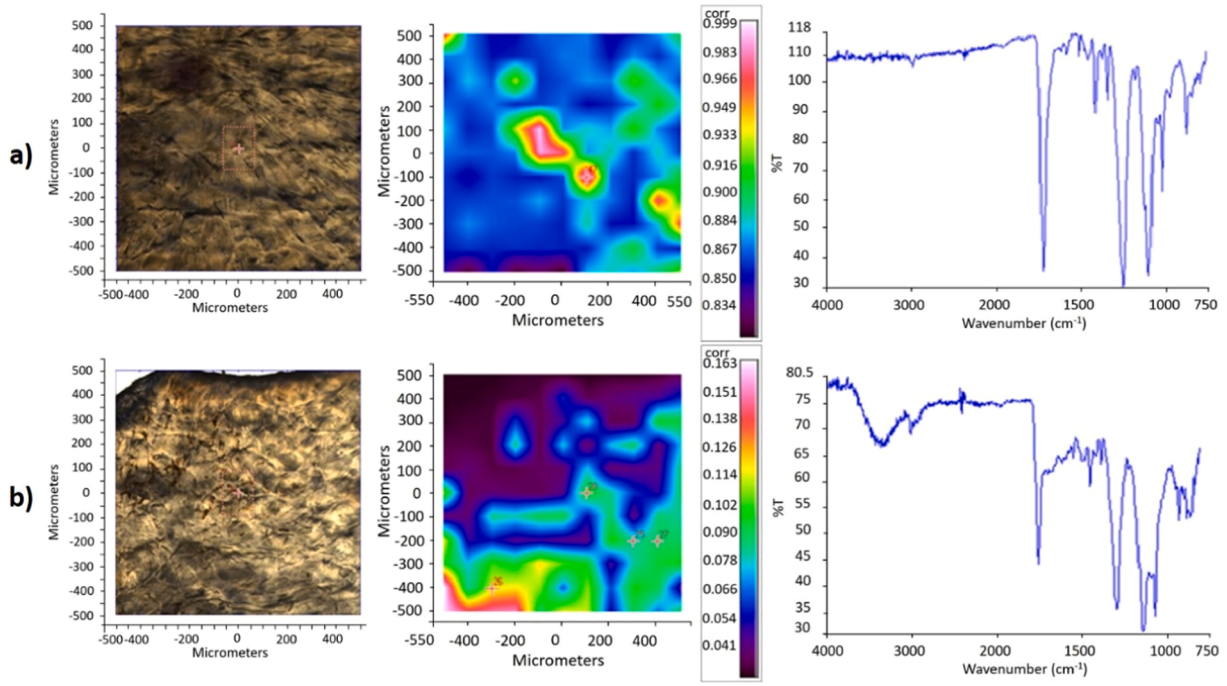

Fig. 7. Chemical Imaging and FTIR analysis results on recycled PET samples untreated (a) and treated with C2 (b): optical images (left), chemical maps (middle), and FTIR spectra by transmittance (right) of the samples' surface area.

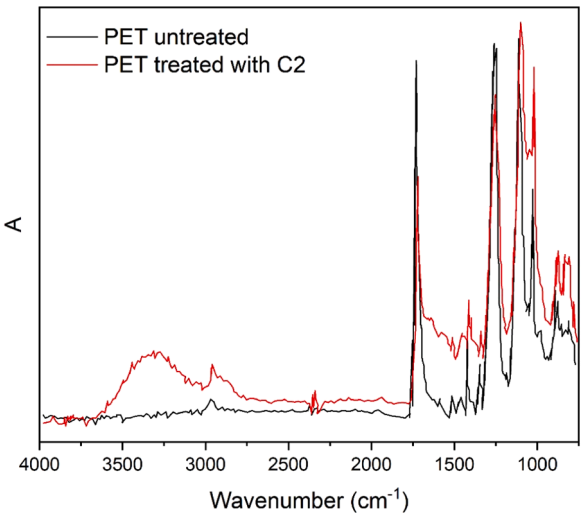

Fig. 8. Comparison of FTIR spectra of recycled PET samples untreated (black) and treated with C2 (red).

To correctly interpret the FTIR results, the Ester Carbonyl Bond Index (ECBI) was calculated as the ratio between the intensity of the signal at 1740 cm-1 and the intensity of the signal at 1465 cm^{-1} (Maheswaran et al., 2023). Since the ECBI is affected by the biodegradation degree, ECBI values before and after the treatment were compared to evaluate the biodegradation of the analyzed polymers. The PET treated with C2 and the untreated PET showed ECBI values of 16 and 14 respectively.

Since the biodegradation degree of PET samples is strongly affected by their crystallinity (Maheswaran et al., 2023), FTIR results were also used to estimate the crystallinity of PET. As reported in the literature, the crystallinity of PET can be determined by the following equation (Maheswaran et al., 2023):

Table 2
Molecular weight of PET treated with C2 in comparison to untreated PET.

Units	Untreated PET	C2 treated PET
[η] (dL/g)	0.683	0.691
M_n	29,584.11	30,060.37
M_w	44,998.45	45,775.50

$$\%Cristallinity = 100 - \left[\left\{ \frac{1 - \frac{I_{1473}}{1.233\ I_{1463}}}{1 + \frac{I_{1473}}{I_{1463}}} \right\} \bullet 100 \right] \quad (1)$$

From results, PET showed a crystallinity of 30 %. FTIR analysis carried out on PET recycled revealed some changes due to the treatment with C2. The slight variations of the Carbonyl Index and the molecular weight values of the samples, before and after the treatment, suggested that the modification of FTIR spectra can be ascribed to the presence of a biofilm on the surface of the PET samples due to the biodegradation treatment. However, the low weight loss registered at the end of the biodegradation could be related to crystallinity degree (30 %). In fact, some authors observed that PET samples with a crystallinity higher than 20 % needed more time to degrade compared to samples with lower crystallinity (Maheswaran et al., 2023).

To further ascertain the impact of C2 treatment on PET, the molecular weight of C2 treated PET was determined by measuring the viscosity in a diluted solution using Standard Method ASTM D 4603. An Ubbelohde-type viscosimeter was used to determine the inherent viscosity of the polymer solution. The solvent used was a mixture of 60 wt% phenol and 40 wt% 1,1,2,2-tetrachloroethane. The results revealed that no significant differences in molecular weight were observed when analysis was performed on pieces of the polymer which comes from the bulk, thus being not assimilated are expected to be not significantly degraded in particular for molecular weight variations (Table 2).

4. Conclusions

The strategic combination of bacterial and fungal strains, based on their enzymatic profile related to plastics metabolism, including ligninolytic, lipolytic and cutinolytic activities, besides the ability to colonise plastics, offers promising results for the development of consortia capable to degrade a broad range of plastic polymers. The consortia C2 (*B. subtilis* RBM2, *F. oxysporum* RHM1, and *A. alternata* RHM4) and C4 (*B. subtilis* RBM2 and *P. alloputida* REBP7) have demonstrated the ability to reduce the weight and modify the molecular structure of plastics such as recycled LDPE, virgin LLDPE and recycled PET. FTIR analysis confirmed effective biochemical interactions with these plastics, showing evidence of initial microbial action on PET, such as the formation of hydroxyl and amino groups. In PE, the presence of carbonyl and hydroxyl groups indicates mild degradation. These results suggest that selected microbial consortia offer scalable and sustainable solutions for plastic waste management, however further research are required to improve their biodegradation efficiency.

CRediT authorship contribution statement

Víctor Carpena-Istán: Methodology, Investigation. **Niccoletta Barbani:** Writing – review & editing, Methodology, Investigation. **Maria J Estrella-González:** Methodology, Investigation. **Ana J Toribio:** Methodology, Investigation. **Maria J López:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Jesús Salinas:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Maria R Martínez-Gallardo:** Writing – review & editing, Methodology, Investigation. **Miriam Cappello:** Writing – review & editing, Methodology, Investigation. **Patrizia Cinelli:** Writing – review & editing, Methodology, Investigation. **Juan A López-González:** Methodology, Investigation. **Macarena M Jurado:** Writing – review & editing, Methodology, Investigation. **Francisca Suárez-Estrella:** Supervision, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Data availability

Data will be made available on request.

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