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MICROBIOLOGY

Biotechnological potential of microorganisms from landfill leachate: isolation, antibiotic resistance and leachate discoloration

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Abstract: Disposal of municipal solid waste (MSW) can be considered a risk to human health representing a great environmental problem in several countries. MSW landfills are a significant source of toxic elements in the environment. Microorganisms able to thriving in leachate wastewater may exhibit metabolic machinery to synthesize a wide range of enzymes able to degrade and/or discolor toxic compounds from leachate. The use of non-pathogenic microbial cells for human health, recovered from leachate for biotechnological application, can be considered a promising approach in bioremediation processes of toxic compounds found in these environments. The present work aimed to the isolation, antibiotic resistance evaluation and leachate discoloration by microorganisms isolated from landfill leachate of Foz do Iguaçu. Forty bacteria and fifteen filamentous fungi were isolated. From these, six bacterial showed resistance at least one tested antibiotic, while six fungal isolates showed resistance to the antimycotic nystatin. CCMIBA_4L (unidentified bacteria) and *Paecilomyces* sp. CCMIBA_5N, were able to discolor 19.15% and 25.26% of the leachate, respectively. The results of the present work encourage future studies to characterize the enzymes involved in the discoloration and degradation of the leachate. The findings demonstrated the potential for the use of microorganisms from landfill leachate as bioremediation tools.

Key words: Microbial resistance, antimicrobial drugs, sanitary importance, urban solid waste.

INTRODUCTION

The technological advances and population growth have contributed to an increase in the formation of municipal solid waste (MSW). This production may significantly increase environmental and public health risks worldwide due to the presence of toxic compounds and potentially pathogenic microorganisms found in these residues (Alfaia et al. 2017, Almeida et al. 2018). MSW from landfills releases a liquid residue of dark color and nauseating odor. This liquid (leachate) is originated from biological, chemical, and physical processes from the organic matter decomposition, may contain organic pollutants, inorganic salts, and heavy metals (Mavakala et al. 2016).

In Brazil, about 59% of MSW was disposed in landfills in 2015, while 41.3% of waste was improperly disposed in controlled or open-air landfills. On the other hand, the collection of recyclable material covers less than half of the national territory. Consequently, recyclable waste is improperly disposed in landfills (Alfaia et al. 2017). In this scenario, the collection and incorrect disposal of garbage generated in the country represent a serious concern about the risks of contamination by pathogenic

microorganisms that municipal waste can provide to people who live near landfills (Kalwasińska & Burkowska 2013, Frączek et al. 2014). Thus, municipal landfill can be considered sources of bioaerosol as well as habitat for insects and rodents responsible for transporting potentially pathogenic microorganisms (Kalwasińska & Burkowska 2013).

Works have emphasized the presence of pathogenic bacteria associated to residues generated in municipal landfills. Kalwasińska & Burkowska (2013) reported the presence of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella, Clostridium perfringens* and coliform in air and soil samples obtained from municipal landfill in Torun, Poland. According to Grisey et al. (2010), total coliforms, *Escherichia coli, Enterococci, Pseudomonas aeruginosa, Salmonella* and *Staphylococcus aureus* were found in groundwater and leachate from aquifer beneath the Etueffont landfill, France. In the same way, pathogenic fungi have been found in the same sampling sites, such as *Aspergillus fumigatus, Cladosporium herbarium, Alternaria alternata* and other airborne microorganisms including *Aspergillus* and *Penicillium* species (Breza-Boruta 2012). Microbial cells may trigger the development of several diseases including allergies, infectious diseases, lung damage, and epidemics (Falencka-Jabłońska & Skorupa 2014). Microbial contamination observed near municipal landfill can be caused by the spread of bioaerosol, birds, rodents, insects, and leachate leaking, mainly in controlled dumps and sanitary landfills, which do not have a leachate waterproofing system (Gouveia 2012, Kalwasińska & Burkowska 2013).

However, microorganisms that thrived in toxic environments can develop a unique metabolic capacity to process these xenobiotic compounds and transformed them into metabolically assimilable and/or less toxic forms. This ability comes from genetic and biochemical adaptation by microbial communities to different toxic chemicals (Bernal et al. 2021). The use of microbial communities recovered from landfill leachate can be considered a promising strategy for application as environmental bioremediation of xenobiotic compounds. The understanding of the pathogenic microbial community associated to leachate from landfills is particularly important for the environment and human health. In this way, the present work evaluated the susceptibility to antibiotics and the leachate discoloration capacity by bacteria and fungi isolated from landfill leachate located in the city of Foz do Iguaçu, for future studies of bioremediation of toxic compounds by using non-pathogenic strains.

MATERIALS AND METHODS

Sampling and isolation

The samples were obtained from landfill leachate located in the city of Foz do Iguaçu (25°27'47.9"S 54°36'26.4"W), in western Paraná State, in two periods, April 2018 and May 2019. The two samples were collected using a sterile 1000 mL glass vial at a depth about 40 cm and were processed by the serial dilution method (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}). Aliquots of 50 µL from each sample diluted were used to inoculate culture medium to isolate filamentous fungi and bacteria. The microbial growth media Potato Dextrose Agar (PDA) (glucose 10 $g.L^{-1}$, agar 15 $g.L^{-1}$, in 1000 mL potato infusion), added chloramphenicol 250 mg L^{-1}, according to Bernal et al. (2021), and Nutrient Agar (NA) (meat extract 3 $g.L^{-1}$, peptone 5 $g.L^{-1}$ and agar 15 $g.L^{-1}$), added nystatin 100.000 U L^{-1}, were used for the isolation of filamentous fungi and bacteria, respectively. The plates were kept at 28 °C and 37 °C for 30 and 15 days for the growth of filamentous fungi and bacteria, respectively. The isolates were preserved in glycerol 20% at -80

°C (Smith & Ryan 2012). The isolates were stored in the Coleção de Cultura de Micro-organismos de Importância Biotecnológica e Ambiental – CCMIBA/UNILA.

Morphological identification

Morphological identification of isolates was performed through macro and microscopic analysis. The strains were cultivated in PDA and NA culture media for seven and two days at 28 °C and 37 °C, for filamentous fungi and bacteria, respectively. The colony colors and growth rates were evaluated in a stereoscope (NIKON SMZ 745 Model C -LEDS - China). The presence and size of fungal sclerotia and conidia morphology were evaluated by the staining method on lactophenol. Bacterial cells were evaluated by use Gram coloration technique. The slides were visualized in an optical microscope (NIKON Eclipse E200MVR - China) (Madigan et al. 2016).

Bacterial biochemical identification

Bacteria were identified by biochemical methods using the culture media CLED Agar (casein peptone 4 $g.L^{-1}$, gelatin peptone 4 $g.L^{-1}$, meat extract 3 $g.L^{-1}$, lactose 10 $g.L^{-1}$, L-cystine 0.128 $g.L^{-1}$, agar 15 $g.L^{-1}$ and bromothymol blue 0.02 $g.L^{-1}$) and MacConkey Agar (peptide casein 1.5 $g.L^{-1}$, meat peptone 1.5 $g.L^{-1}$, gelatin peptone 17 $g.L^{-1}$, bile salts 1.5 $g.L^{-1}$, lactose 10 $g.L^{-1}$, sodium chloride 5 $g.L^{-1}$, neutral red 0.03 $g.L^{-1}$, crystal violet 0.001 $g.L^{-1}$, and agar 13.5 $g.L^{-1}$). The catalase assay was performed by addition of H_2O_2 in bacterial colonies. To confirm the genus *Bacillus*, the malachite green dye was used to verify the presence of spores (Levy 2004).

Antibiogram assay

All bacteria and fungi strains were evaluated for their resistance against antimicrobials according to Kirby-Bauer diffusion method (Laborclin 2011). The bacterial isolates were transferred to test tubes containing 3 mL of distilled H_2O sterilized. All experiments were carried out in triplicates and with OD standardized for 0.08. Swabs (sterilized) were placed in this solution and were used to seed plates with the Mueller-Hinton Agar (MH) (beef extract 2 $g.L^{-1}$, acid hydrolysate of casein 17.5 $g.L^{-1}$, starch 1.5 $g.L^{-1}$ and agar 17 $g.L^{-1}$) (Levy 2004). Solutions of the commercial broad-spectrum antimicrobials including amoxicillin + potassium clavulanate (500 + 125 $mg.L^{-1}$), azithromycin (500 $mg.mL^{-1}$) and chloramphenicol (250 $mg.L^{-1}$), were prepared. Sterile pieces of disc filter paper (5 mm diameter), soaked for 1 minute in each antibiotic solution (3 pieces in each drug) were added to the plates (MH) with striated bacteria. As a control, three disks embedded with Lysoform® were used. The plates were incubated for 24 - 48 hours at 37 °C. The formation of halos without microbial growth around each disc was considered a result of drug sensitivity.

The filamentous fungi were cultivated on PDA, added nystatin 100.000 $UI.L^{-1}$. Discs of 5 mm in diameter, obtained from the margins of the colonies, were inoculated onto PDA plates (in triplicate) for 7 days at 28 °C. As a control, PDA medium was used without nystatin. The presence of microbial growth in the assay was considered a result of drug resistance (Alastruey-Izquierdo et al. 2015), modified.

Leachate discoloration assay

The ability of microbial strains to discolor leachate in liquid culture medium was performed according to da Silva et al. (2008) modified. All isolates were grown on PDA and NA media for fungi and bacteria, respectively. One fungal culture disc (5 mm diameter) from the edge of the colony and one bacteria colony, were transferred to flasks containing leachate as the only nutrient source. The flasks were incubated in shaker at 150 rpm for five and two days at 28

°C and 37 °C, for fungi and bacteria, respectively. Aliquots of 2 mL were collected, centrifuged at 12.000 rpm for 2 minutes. The reduction of absorbance was verified in Spectrophotometer at 450 nm. (Makhatova et al. 2020). Flasks containing leachate free of cells were used as control. All assays were conducted in triplicate. The efficiency of discoloration was expressed by the formula:

$$\text{Decolorization (\%)} = \frac{A_{\lambda,\ \text{initial}} - A_{\lambda\text{final}}}{A_{\lambda,\ \text{initial}}} \times 100$$

$A_{\lambda\ \text{initial}}$ = initial absorbance and $A_{\lambda\ \text{final}}$ = final absorbance

RESULTS AND DISCUSSION

The isolation of microbial strains from landfill leachate recovered 9 filamentous fungi and 21 bacteria from the first sampling (April 2018), and 6 filamentous fungi and 19 bacteria from the second sampling (May 2019). The number of bacterial isolates (n= 40) recovered was higher compared to the fungal isolates (n=15). All bacteria isolates were submitted to microscopic and biochemical analysis to identify the taxonomic groups present in the samples. The analysis revealed two distinct groups, Gram-positive and Gram-negative bacteria including bacilli (rod-shaped), cocci (spherical-shaped) and coccobacilli (shaped like very short rods or ovals), being 17 different morphotypes from the two sampling, 9 and 8 from the first one and the second, respectively. Thirty-eight (95%) of the isolates were identified as catalase-positive and all isolates (100%) as non-lactose fermenting (Table I).

The most abundant morphotype recovered from the samples collected in 2018 was morphotype 5, Gram-positive bacilli with matte cream colony characteristics (n=6). On the other hand, two morphotypes were the most abundant from the sample collected in 2019, including morphotype 10 (yellow colony Gram-positive streptobacilli, n=4) and the morphotype 16 (matte green colony Gram-positive staphylococci, n=4). Was possible to observe the same morphotype (2 and 11) which appeared in the two sampling (2018 and 2019), represented by a bright yellow colony, Gram-positive cocci (Table I). The genera identified were *Bacillus* sp. (n=8) recovered from two sampling, *Staphylococcus* (n=6), *Streptococcus* (n=4), representatives from Firmicutes_phylum as well as, *Pseudomonas* sp. (n=1), from Proteobacteria phylum and several not identified (N.I.) isolates. The analysis from filamentous fungi isolated revealed three distinct groups affiliated with species from genera *Aspergillus,* including *A. niger* (n = 1) and *A. fumigatus* (n= 2), *Paecilomyces* sp. (n=1), *Curvularia,* including *Curvularia* spp. (n= 5), *C. lunata* (n= 1) and *C. inaequalis* (n= 1), and four isolates affiliated with non-morphologically identified taxonomic groups (Table II).

Reports in the literature have described the isolation and/or presence of microbial cells in landfill leachate including bacteria and fungi such as *Pseudomonas aeruginosa, P. fluorescens, Pseudomonas* sp., *Staphylococcus aureus, S. xylosus, S. hominis, S. warnerii, Streptococcus, Aspergillus* sp., *Penicillium* sp., *Mucor* sp., and *Fusarium* sp. (Aicha et al. 2013, Borquaye et al. 2019, Zegzouti et al. 2020, Shi et al. 2021). The fungal taxonomic groups recovered from the leachate samples in the present study, have already been recovered in other studies of isolation and evaluation of biotechnological potential of microorganisms derived from MSW. Gautam et al. (2012) conducted an isolation study of filamentous fungi in samples from Municipal solid waste in Jabalpur, India. The

Table I. Bacteria strains isolated from leachate and resistant potential to commercial antibiotics.

Code (CCMIBA)	Resistance				MacConkey (growth)	Agar CLED	Catalase	Ferm. Lactose	Morphology / Gram	Identification
	Control (Lysoform®)	Amox. +Clav. Ac	Azi.	Cloranf.						
Bacteria (sampling April 2018)										
1L 4L 7N	S	S	S	S	+	**Morphotype 1:** matte yellow colony with red end	+	-	Diplococci/ -	N.I.
1M 12N	S	S	S	S	-	**Morphotype 2:** bright yellow colony	+	-	Cocci/ +	N.I.
1N 3M	S	S	S	S	-	**Morphotype 3:** bright pink colony	+	-	Bacilli/+	N.I.
9M 2M	S	S	S	S	+	**Morphotype 4:** bright pink colony	+	-	Bacilli/ -	N.I.
2L	S	**R**	**R**	S	-	**Morphotype 5:** matte cream colony	+	-	Bacilli/+	*Bacillus* sp.
3L		S	S							
6N		R	R							
7L			S							
7.2L										
7.1N										
2.1N	S	**R**	**R**	S	+	**Morphotype 6:** green colony	+	-	Bacilli/ -	*Pseudomonas* sp.
8L		R	R							
8.9M 8N	S	S	S	S	-	**Morphotype 7:** cream colony	+	-	Cocci/+	N.I.
9L	S	S	S	S	-	**Morphotype 8:** cream colony	+	-	Bacilli/+	N.I.
7M	S	S	S	S	-	**Morphotype 9:** bright cream yellow colony	+	-	Bacilli/+	*Bacillus* sp.
Bacteria (sampling May 2019)										
B1	S	S	S	S	-	**Morphotype 10:** yellow colony	+	-	Staphylococci/+	*Staphylococcus* sp.
B3		R	R							
B4		S	S							
B9		S	S							
B7		S	S							
B8		S	S							
B5 B10	S	S	S	S	-	**Morphotype 11:** bright yellow colony	+	-	Cocci/+	N.I.
B2 B12 B13	S	S	S	S	+	**Morphotype 12**: bright green colony	+	-	Cocci/-	N.I.

Table I. Continuation.

B6	S	S	S	S	-	**Morphotype 13:** matte green colony (small)	-	-	Coccobacilli/+	N.I.
B11	S	S	S	S	-	**Morphotype 14:** bright yellow colony (small)	-	-	Cocci/+	N.I.
B14	S	S	S	S	+	**Morphotype 15:** greenish yellow colony	+	-	Cocci/-	N.I.
B15	S	S	S	S	-	**Morphotype 16:** matte green colony	+	-	Streptobacilli/+	*Streptococcus* sp.
B16										
B17										
B18										
B19	S	S	S	S	-	**Morphotype 17:** cream colony	+	-	Bacilli/+	*Bacillus* sp.

R = resistant. S = sensitive. N.I. = not identified.

results of the study showed that among 250 isolates, representatives from taxonomic groups including *Aspergillus niger*, *Curvularia lunata*, *Curvularia* sp. and *Paecilomyces* sp., were recovered.

Landfills are considered reservoirs for many pharmaceutical products, providing a favorable habitat for microbes resistant to antimicrobials and transfer of resistant genes between microbial cells (Borquaye et al. 2019). Concerning antibiotic sensitivity, eight strains (20%) showed resistance at least one of the assayed antibiotics. From these, five strains were resistant to amoxicillin + potassium clavulanate and azithromycin including *Bacillus* (n=5), *Pseudomonas* (n=2) and *Staphylococcus* (n=1) genera. On the other hand, none strain showed resistance to chloramphenicol (Table I). *Bacillus*, *Pseudomonas* and *Staphylococcus* genera have already been reported in the literature as strains recovered from landfill samples which showed antibiotic resistance as well as genes responsible for this resistance (Efuntoye et al. 2011, Borquaye et al. 2019). *Pseudomonas aeruginosa* species is an opportunistic agent frequently involved in nosocomial infections and drug resistance situations (da Mata & Abegg 2013). Data from the Health Surveillance Secretariat, between 1999 and 2008, 6.062 outbreaks of foodborne illnesses were recorded in Brazil, with approximately 117.000 people affected. *Bacillus cereus* appeared in third place as causer agent for outbreaks, being responsible for 205 of these cases, followed by *Staphylococcus aureus*, which caused 600 outbreaks (Brasil 2010). Thus, the presence of species resistant to commercial antibiotics including amoxicillin + potassium clavulanate and azithromycin, compounds present in leachate from municipal sanitary landfills, becomes a major public health problem.

Likewise, it was possible to observe that from 15 fungal strains isolated, 40% (n = 6) including the strains *Aspergillus fumigatus* (n=2), *Curvularia lunata* (n=1), *Curvularia* sp. (n=1) and not identified (n=2) were resistant to antibiotic nystatin (Table II), in other words, there was microbial growth in the culture medium supplemented with nystatin. Reports in the literature have already been demonstrated the fungal resistance to drug nystatin. However, it was results that evaluated the resistance to

this drug using clinical fungal strains including *Candida* spp., isolated from patients or animals (Farias et al. 2003, Chokoeva et al. 2016, Wiederhold 2017). The antifungal susceptibility profile of *Aspergillus fumigatus* strains recovered from lungs of birds was evaluated in the study performed by Spanamberg et al. (2020). The authors tested fifty-three isolates for their antifungal susceptibility to the drugs voriconazole, itraconazole, amphotericin and caspofungin. Most isolates were resistant at least one antibiotic assayed.

Curvularia species are considered pathogens that cause disease in plants and humans, with the development of mild, febrile, and potentially fatal illnesses if not well treated (Bengyella et al. 2017). In the same way, *Aspergillus fumigatus* can cause Aspergillosis, a disease that can present itself in an allergic, saprophytic, or invasive way. One of the aspergilloses of concern is an allergic bronchopulmonary disease, characterized by corticosteroid-dependent asthma, fever, hemoptysis, and airway destruction, which can progress to fibrosis (Sales 2009). Thus, the use of effective drugs to fight against infectious strains becomes more and more necessary.

The landfill receives unused and unwanted antibiotics through household waste. Thus, the existence of antibiotic resistance genes in these environments is a fact, which makes the landfill an important reservoir of resistance bacteria (Wang et al. 2015, Shi et al. 2021). Antibiotic resistance genes have been detected in several environments such as sediments (Luo et al. 2010), river (Garcia-Armisen et al. 2011, Luo et al. 2010), effluent, sewage treatment plants (Chen & Zhang 2013, Munir et al. 2011), and soil from pig farms (Wu et al. 2010). However, they have rarely been characterized in landfills or leachate (Wang et al. 2015).

Studies emphasizing the resistance of fungi isolated from landfills to antimycotic action of certain drugs are very rare. Amani et al. (2018) performed a study where the antifungal susceptibility of *Candida* spp. isolated from landfill leachate in Borj Chakir, Tunisia. The results showed that 12 strains, from 37 isolates recovered from the samples, showed resistance to antibiotic Amphotericin B. Thus, our study can be considered the first report on microbial resistance of *A. fumigatus* and *Curvularia* spp. including *C. lunata*, to the antimycotic nystatin.

Table II. Fungi strains isolated from leachate and resistant potential to nystatin.

Code (CCMIBA)	Resistance (nystatin)	Macro and Micro Morphology
Filamentous fungi (sampling April 2018)		
1C	S	N.I.
1M	**R**	
1N	**R**	*Aspergillus fumigatus*
2M	**R**	
3E	S	*Aspergillus niger*
3N	S	N.I.
4C	R	
5M	S	*Curvularia* sp.
5N	S	*Paecilomyces* sp.
Filamentous fungi (sampling May 2019)		
F1	S	*Curvularia* sp.
F2	S	
F3	S	*Curvularia inaequalis*
F4	**R**	*Curvularia lunata*
F5	S	*Curvalaria* sp.
F6	R	

N.I.: not identified. R = resistant. S = sensitive.

To reduce the adverse effects of landfill leachates on the environment, aerobic treatments have been widely used for the treatment of leachates, with a high removal efficiency of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and discoloration reduction (Mrabet et al. 2020). Discoloration analysis is an important method for selecting microorganisms. When microorganisms use the compounds present in the pollutant as a source

of nutrients, the microorganisms degrade the colored compounds, indicating their biochemical breakdown. In the leachate, the pollutant responsible for the color can be divided into four main groups: *i)* dissolved organic matter composed of volatile fatty acids and refractory compounds similar to fulvic and humic compounds; *ii)* inorganic macro components formed by calcium, magnesium, sodium, potassium, ammonium, iron, manganese, chloride, sulfide, and hydrogen carbonate; *iii)* heavy metals composed of cadmium, chromium, copper, lead, mercury, nickel, zinc; and iv) xenobiotic organic compounds present in low concentrations (less than 1 $mg.L^{-1}$), including aromatic hydrocarbons, phenols, aliphatic chain chlorines, pesticides, and plasticizers (Di Iaconi et al. 2006, Kjeldsen et al. 2002).

The present work evaluated the microbial growth in the landfill leachate, as the only nutrient source, and its discoloration using microbial cells recovered from the landfill. All isolates, 40 bacteria and 15 filamentous fungi, were subjected to leachate discoloration assay. Of the total, twelve bacteria (30%) were able to discolor leachate, with the percentage of discoloration ranging from 3.29% to 25.26%, with the strain CCMIB_4L (not identified) being the most efficient in discoloring leachate. Concerning filamentous fungi, eight isolates (53%) were able to discolor leachate, with the percentage of discoloration ranging from 2.76% to 19.15%. *Paecilomyces* sp. CCMIBA_5N was the most efficient in discoloring leachate (Table III). Both strains were sensitive to antibiotics assayed (Table I and II), showing security for future studies in biodegradation of the compounds from leachate.

Few studies described in the literature performed the discoloration of leachate using microorganisms recovered from this toxic environment. The vast majority used non-biological processes to treat leachate from landfills including coagulation-flocculation, advanced oxidation technologies, precipitation, ion exchange, membrane filtration and

Table III. Discoloration of leachate as the only source of nutrient.

Strain (CCMIBA)	Discoloration (%) (2 days of growth)	Strain (CCMIBA)	Discoloration (%) (5 days of growth)
Bacteria		**Filamentous fungi**	
N.I. 4L	**25.26**	N.I. 1M	7.05
Bacillus sp. 3M	10.01	N.I. 3N	2.76
Pseudomonas sp. 2.1N	6.69	N.I. 4C	6.24
Staphylococcus sp. B3	5.29	*Curvularia* sp. 5M	17.62
N.I. B5	7.05	*Paecilomyces* sp. 5N	19.15
N.I. B12	3.29	*Curvularia* sp. F1	7.8
N.I. B6	16.38	*Curvularia* sp. F5	3.72
Staphylococcus sp. B8	11.49	N.I. 1C	12.24
N.I. B11	7.96		
N.I. B14	8.41		
Streptococcus sp. B18	3.29		
Bacillus sp. B19	6.28		

N.I.: not identified.

adsorption (Chaouki et al. 2017a, b, Cossu et al. 2018, Mrabet et al. 2020, Reynier et al. 2015). The studies that used biological pre-treatments as a tool to improve physicochemical treatments, did not described the genera and/or microbial species used, as well, it did not mention whether the microbial cells were recovered or not from the leachate and used in the pre-treatment (Quraishi et al. 2019).

Mrabet et al. (2020) applied anaerobic treatment using a bioreactor to treat young leachate generated in the landfill of Fez city, Morocco. After three days, the authors achieved a 50% reduction in leachate color. Elleuch et al. (2020), performed a study using the product Kefir grains as a pre-treatment, to remove toxic pollutants from Jebel Chakir landfill leachate, Tunis city, Tunisia. Kefir grains are a complex symbiotic association of bacterial and yeasts present in an exopolysaccharide matrix. The microorganisms present in this complex including species of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*, *Acetobacter*, *Kluyveromyces*, *Candida,* and *Saccharomyces* (Dertli & Çon 2017). The authors found, after five days of pre-treatment, the removal rates of TOC, COD, NH_4^+ -N and PO_4^{3-} were 93, 83.33, 70 and 88.25%, respectively. In the same way, other studies have reported in the literature, about the treatment of solid urban waste and/or the removal of metals found in leachate, using fungal cells isolated from leachate samples. Awasthi et al. (2017), evaluated the potential of indigenous fungi including *Trichoderma harzianum*, *Aspergillus niger* and *Aspergillus flavus* for biosorption of Cd^{2+} from leachate. The study demonstrated a promising solution for removing metals from municipal solid waste leachate. Gautam et al. (2012), evaluated the biodegradation of organic urban solid waste using filamentous fungi recovered from samples of different substrates, including municipal solid waste, compost, and soil. The study was conducted with a *Trichoderma viride* strain, using waste piles. The authors observed biodegradation (average weight loss) of 33.35% of organic waste from piles after 60 days.

Results obtained in the present work demonstrated the existence of microorganisms potentially pathogenic present in leachate from municipal landfill as well as the microbial resistance of these strains to commercial antibiotics, which raises a major public health concern. However, was demonstrated the biotechnological potential that microbial communities recovered from landfill leachate may present. Fungi and bacteria inhabiting this environment can produce compounds able to be used in the bioremediation processes of leachate. Further research needs to be carried out to identify and characterize the compounds possibly metabolized as well as the microorganisms responsible for the discoloration of leachate to be used in *situ* bioremediation processes.

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LAAG, JERM, DBVB, and MRZP carried out the analyses of the data; RCBS and MRZP wrote, reviewed and edited the manuscript.