

The Microbiome of olive mill wastes

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Abstract

Olive mill wastes (OMWs) pose a significant environmental threat due to their high-strength organic nature, which can harm soil and water quality and have adverse effects on aquatic and terrestrial ecosystems. This review paper aims to present an updated understanding of the microbial communities found in OMWs over the past two decades, employing both culture-dependent and independent approaches. Through a comprehensive survey of 16S rRNA gene sequences from various olive mill waste environments, researchers have identified the prevalence of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, and Actinobacteria. The alarming detection of fecal bacteria and human pathogens in OMWs raises considerable concern and calls for further investigation. Although previous research has primarily focused on using known bacterial and fungal species from different environments for olive mill waste degradation, the untapped biotechnological potential of native microbiota demands greater exploration for the bioremediation of OMWs and the eradication of plant and human pathogens.

Keywords: Olive Mill Waste (OMW); Degradation and Detoxification; Bacterial and fungal species; Bioremediation

1. Introduction

The disposal of olive mill wastes presents a significant environmental challenge in olive-oil producing countries, as these wastes are generated in large quantities within a short timeframe. While olive oil production is predominantly concentrated in Mediterranean countries, other producers like Argentina, Australia, and Portugal also grapple with the toxic effects of olive mill wastes. The major olive oil producers include Spain, Italy, and Greece, with annual productions of 1150, 560, and 370 thousand tons, respectively, followed by Tunisia and Turkey, each producing approximately 160 thousand tons per year. In the olive oil extraction process, two and three-phase centrifugal decanters are commonly utilized, with the two-phase extraction system gaining popularity due to its lower water consumption during olive waste malaxation. Three-phase extraction systems yield olive oil, olive press cake, and a liquid waste known as olive mill wastewater (OMW), while two-phase decanters produce olive oil and a viscous sludge-like waste called "alpeorujo" in Spanish, abbreviated as TPOMW (two-phase olive mill waste). Both types of wastes, OMWs and TPOMW, share common undesirable characteristics such as unappealing color and odor, acidic pH levels, high salt concentration, and significant content of total polyphenolics. Additionally, OMWs are distinguished by their elevated chemical oxygen demand (COD) values, signifying the presence of substantial organic pollutants, while TPOMW is characterized by high organic matter content and low water activity. The physico-Chemical properties of OMW characteristic value ranges for PH is 4.01-5.93 and TPOMW is 4.86-6.45. For total phenolics it ranges from 0.03-18.9 for OMW and 0.5-2.4 for TPOMW. (1 Based on data reported in Aktas et al. [6], Ammary [7], Zenjari et al. [8], Amaral et al. [9], Eroglu et al. [10], Aviani et al. [11], and Ntougias et al. [12]. 2 Based on data reported in Albuquerque et al. [13] and Baeta-Hall et al. [15]). Olive mill wastes pose substantial environmental pollution concerns due to their high organic load, elevated levels of salt, and polyphenols content. These wastes can hinder seed germination and early plant growth, leading to alterations in soil properties and creating reducing conditions that impact soil microbial diversity. On the other hand, the phenolics present in olive mill wastes hold potential applications in the food and chemical industries as natural antioxidants and disinfectants, offering

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sustainable alternatives. Furthermore, these wastes can be utilized to produce biopolymers and biogas, which can support the cultivation of edible fungi, contributing to more sustainable and eco-friendly practices.

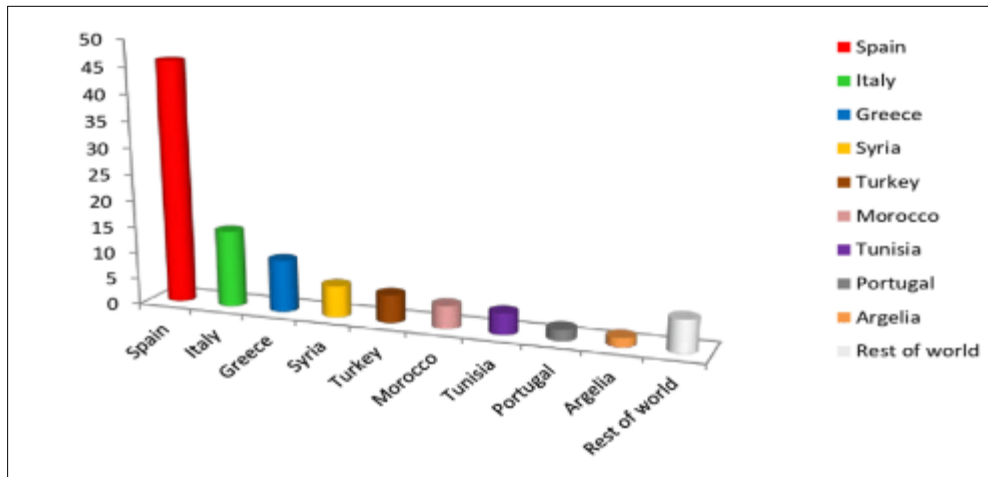


Figure 1 Main Olive oil Producing Countries

Numerous treatment methods, encompassing both physicochemical and biological approaches, have been employed to degrade and detoxify olive mill wastes. Some of these methods include advanced oxidation systems, aerobic biotreatment, and anaerobic digestion. However, it is essential to note that this paper's scope does not encompass an exhaustive review of all treatment technologies for both OMW and TPOMW.

In this review, the microbiology of olive mill wastes is examined well and special focus is given to topics;

- The microbial ecology of olive mill wastes
- OMW-induced Toxicity
- The effects of olive mill wastes on soil microbial Communities
- The microbial ecology in bioreactors treating olive mill effluents and
- The potential biotechnological application of olive mill waste microbiota.

2. Microbial Ecology of Olive Mill Wastes

2.1. Bacterial Diversity in Olive Mill Wastes

The majority of microbiota found in OMWs originate from soil and freshwater environments, and fecal bacteria have also been detected. The bacterial community structure is significantly influenced by the specific olive cultivar from which the OMWs are generated. Interestingly, when examining OMW samples from different olive-fruit varieties, only 15% of the operational taxonomic units (OTUs) were found to be shared, highlighting a cultivar-dependent microbial profile. In a study conducted by Tsiamis et al., the cultured bacterial diversity in all OMW samples consisted of members from *Firmicutes*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Bacteroidetes*. However, when a high-density DNA microarray (PhyloChip) was employed, a more diverse microbial profile was revealed, dominated by members of all classes of *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Chloroflexi*, *Cyanobacteria*, and *Actinobacteria*. The bacterial phyla *Acidobacteria*, *Planctomycetes*, *Gemmatimonadetes*, *Verrucomicrobia*, and candidate divisions OP3, TM7, AD3, marine group A, and SPAM were found to be minor components of the bacterial biota in the studied samples. The development of DNA microarrays revolutionized microbial analysis, enabling researchers to rapidly and comprehensively detect microbial sequences from any sample in a parallel and high-throughput manner. Pioneering studies conducted by Tsiamis et al. and Goberna et al. utilized these advanced approaches to study the bacterial communities (using PhyloChip) and archaeal communities (using ANAEROCHIP to study methanogenic archaea) in OMW and anaerobically digested TPOMW, respectively.

The bacterial community structure in OMW is significantly influenced by cultivation and harvesting practices. In OMW derived from the "Mastoidis" variety, bacterial diversity was predominantly composed of fermentative members, including lactic acid bacteria (*Lactobacillus* and *Oenococcus spp.*) and acetic acid bacteria (*Acetobacter* and *Gluconacetobacter spp.*), along with fecal bacteria associated with the family *Prevotellaceae* and the *Ruminococcus-*

Eubacterium-Clostridium (REC) cluster. The proliferation of this particular bacterial community structure can be attributed to the accumulation of olives in the harvest net, creating anaerobic or microaerophilic conditions, which are conducive to the growth of these fermentative bacteria. During processing in olive mills, the oxygen levels increase, favoring the growth of acetic acid bacteria. In OMW generated from *O. europaea* var. *koroneiki*, various bacterial families within the phyla Betaproteobacteria, Gammaproteobacteria, and Firmicutes were identified. These included *Comamonadaceae*, *Gallionellaceae*, *Hydrogenophilaceae*, *Methylophilaceae*, *Oxalobacteraceae*, and *Rhodocyclaceae* from Betaproteobacteria, *Pasteurellaceae* and *Xanthomonadaceae* from Gammaproteobacteria, and *Bacillaceae*, *Paenibacillaceae*, *Peptococcaceae*, and *Sporolactobacillaceae* from Firmicutes. On the other hand, Vivas et al. found that TPOMW was mainly dominated by members of the phylum Proteobacteria, with *Actinobacteria (Streptomyces)* and *Firmicutes (Staphylococcus)* present as minor constituents of the olive waste microbiota. Within Gammaproteobacteria, members of *Hydrocarboniphaga*, *Pseudoxanthomonas*, and *Stenotrophomonas* were identified, while the Betaproteobacteria group *Comamonas* was the primary microbial group detected. Additionally, among Alphaproteobacteria, only a single representative, *Brevundimonas sp.*, was found in TPOMW. To address the disposal challenges associated with acidic pH and undesirable odor in TPOMW, the addition of $\text{Ca}(\text{OH})_2$ leads to the formation of alkaline TPOMW, creating a more favorable environment for alkalitolerant and alkaliphilic bacteria, some of which exhibit halophilic traits. Within alkaline TPOMW, halotolerant alkaliphiles related to *Bacillus*, *Idiomarina*, *Halomonas*, and *Nesterenkonia* genera have been identified, along with alkalitolerant and/or halotolerant bacteria phylogenetically associated with *Corynebacterium*, *Novosphingobium*, *Ochrobactrum*, *Pseudomonas*, *Rhodobacter*, and *Serratia*. Many of these isolates have demonstrated the effective utilization of phenolic compounds as their sole carbon source. Notably, the microbiota of olive pomace appear to adapt their membrane lipids in an atypical manner to cope with stressful conditions, such as the reduced water activity (a_w), low acidity, and high polyphenolic content present in olive mill wastes. However, it is essential to consider that *Staphylococcus* spp. found in olive mill waste should be regarded as potential infectious agents, warranting attention and appropriate management measures. Based on microbial counts and API identification, *Enterobacter cloacae* strains were frequently observed in the raw effluent of olive mill wastes, followed by *Pseudomonas aeruginosa* and *Serratia odorifera*. During acidogenesis, *Citrobacter braakii* was found to be predominant. Additionally, high counts of *Acinetobacter*, *Pseudomonas*, and *Enterobacter* spp. have been determined in both OMW and TPOMW. Notably, *Enterobacter* spp. have the potential to act as human pathogens, raising concerns about their presence in olive mill wastes. The analysis of 16S rRNA gene sequences deposited in international databases, despite possible biases due to PCR amplification or the specific focus of research work (e.g., tannase-expressing communities), revealed the presence of 585 bacterial sequences identified in olive mill waste environments. These sequences indicate that the main bacterial representatives in olive mill wastes, including both OMW and TPOMW, belong to the phyla Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, and Actinobacteria. Notably, Betaproteobacteria and Gammaproteobacteria are most prevalent in these environments. Firmicutes, Alphaproteobacteria, Actinobacteria, and Bacteroidetes collectively account for approximately 45% of the bacterial phlotypes identified in olive mill wastes.

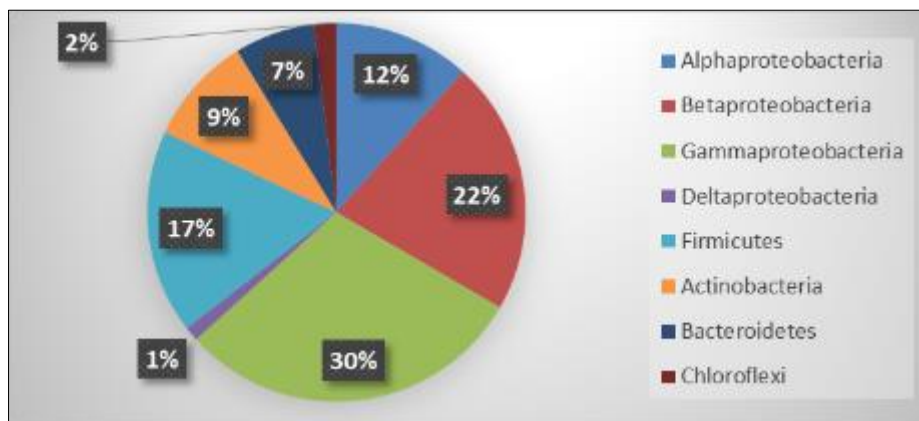


Figure 2 Overall Bacterial Phlotypes in OMW Environment

In olive mill wastes, the predominant Betaproteobacteria belong to the families *Oxalobacteraceae* and *Comamonadaceae*, while the dominant taxon within Alphaproteobacteria is *Acetobacteraceae*. Among the phylum Firmicutes, the most abundant taxa in olive mill wastes are *Bacillaceae*, *Clostridiaceae*, *Lactobacillaceae*, and *Paenibacillaceae*. Actinobacterial phlotypes mainly fall within the families *Micrococcaceae*, *Microbacteriaceae*, and *Propionibacteriaceae*. Bacteroidetes phlotypes identified in olive mill wastes are associated with the families *Prevotellaceae*, *Porphyromonadaceae*, and *Sphingobacteriaceae*. These findings indicate that olive mill waste environments are characterized by bacterial taxa

specialized in degrading the recalcitrant components present in olive mill wastes. Notably, approximately 20% of the bacterial phylotypes identified in olive mill wastes and related environments are associated with coliforms, such as *Citrobacter*, *Escherichia*, *Klebsiella*, and *Serratia spp.*, and other enteric bacteria, including *Porphyromonadaceae*, *Prevotellaceae*, *Lachnospiraceae*, *Eubacteriaceae*, *Peptococcaceae*, *Peptostreptococcaceae*, and *Ruminococcaceae spp.*

2.2. Fungal Diversity in Olive Mill Wastes

The yeast population in olive mill waste appears to be abundant. Recently, various yeast species have been isolated from OMW, including *Geotrichum candidum*, *Candida membranifaciens*, *C. michaelii*, *C. inconspicua*, *C. tropicalis*, *Pichia fermentans*, *P. holstii*, and *Saccharomyces cerevisiae*. In Italian OMW, *Candida boidinii*, *Pichia holstii* (syn. *Nakazawaea holstii*), *P. membranifaciens*, and *Saccharomyces cerevisiae* were found to be the predominant yeasts, exhibiting significant pectolytic and xylanolytic activities. These yeast isolates effectively reduced total phenolics and specifically reduced several phenolic compounds, including p-coumaric, vanillic, and caffeic acids. Similarly, in OMW from Moroccan olive mills, the main yeast biota comprised of *Pichia guilliermondii* (syn. *Meyerozyma guilliermondii*) and *Candida diddensiae* and *C. ernobii* species. These findings highlight the presence of diverse and active yeast populations in olive mill wastes, contributing to the degradation of organic compounds and phenolic reduction processes.

In TPOMW, the predominant yeast taxa were *Pichia caribbica* (syn. *Meyerozyma caribbica*), *P. holstii* (syn. *Nakazawaea holstii*), and *Zygosaccharomyces fermentati* (syn. *Lachancea fermentati*), while minor yeast constituents included *Z. florentinus* (syn. *Zygorhynchus florentinus*), *Saccharomyces cerevisiae*, and *S. rosinii* (syn. *Kazachstania rosinii*). Several yeast isolates from TPOMW were found to exhibit various enzymatic activities, including cellulase, β -glucanase, glucosidase, peroxidase, and polygalacturonase. These enzymatic capabilities suggest their potential contribution to the degradation of complex compounds, including phenolics present in olive pomace. Moreover, in OMW disposal ponds, various fungal genera, such as *Acremonium*, *Alternaria*, *Aspergillus*, *Chalara*, *Fusarium*, *Lecytophthora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phycomyces*, *Rhizoglyphus*, and *Scopulariopsis*, have been identified. These fungi possess the ability to detoxify olive mill effluents, further contributing to the microbial diversity and potential bioremediation processes in olive mill waste environments. The identification of fungi mentioned earlier was primarily based on their morphology and not on molecular techniques. However, there are indigenous microbiota, such as *Cerrena*, *Byssosclama* (syn. *Paecilomyces*), *Lasiodiplodia*, and *Bionectria*, that have been identified through molecular techniques and demonstrate the ability to degrade phenolics in OMW. Among the yeasts found in olive mill wastes, *Pichia*, *Candida*, and *Saccharomyces*-like species are predominant. These yeasts play a key role in the reduction of both phenolics and sugars in olive mill wastes, though they appear to contribute less to OMW decolorization when compared to white-rot fungi. Filamentous fungi, including *Aspergillus* and *Penicillium spp.*, are commonly found in olive mill wastes, while white-rot fungi are isolated to a lesser extent. The unique characteristics of olive mill wastes, such as high salt and sugar concentrations, as well as acidic pH, seem to favor the growth of osmotolerant yeasts in these environments.

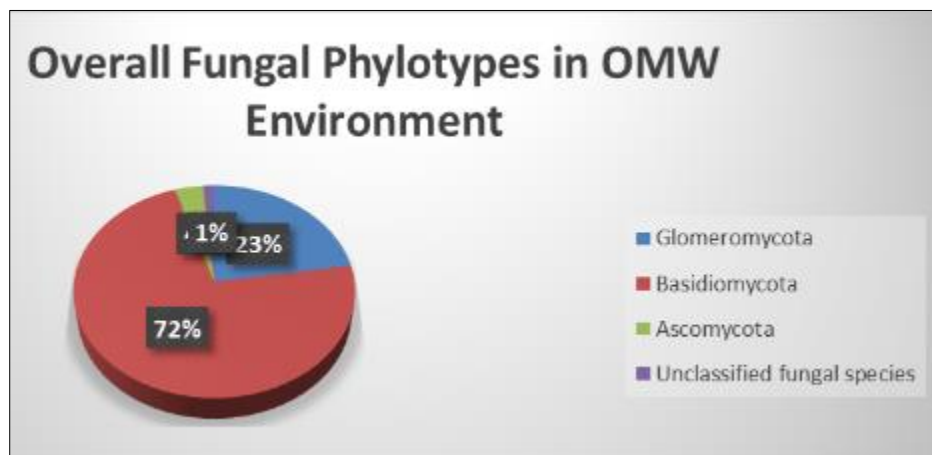


Figure 3 Overall Fungal Phlotypes in OMW Environment

3. OMW-Induced Toxicity

Olive mill wastes (OMWs) pose toxicity to both microorganisms and aquatic organisms. Traditional mills produce OMWs with higher toxicity due to their more concentrated effluents compared to continuous extraction systems. OMW effluents negatively impact aquatic fauna in fluvial ecosystems due to their high organic load and fecal contamination. Raw OMW has been characterized as "extremely toxic" based on Thamnotox kit and zebrafish embryo tests, and even

after biotreatment, they retain a significant level of toxicity. *Aliivibrio fischeri* bioluminescence assays indicate that OMW toxicity can reach maximum levels. The phenolic fraction of olive mill wastes has been found to exhibit potent antimicrobial activity against nonindigenous strains of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The antimicrobial effect is even greater than the activity induced by individual phenolic compounds, suggesting a synergistic action of olive mill waste phenolics. Interestingly, experimental data reveal that individual phenolic compounds at low concentrations cannot inhibit the growth of human pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. However, OMWs exhibit robust inhibitory effects against both Gram-positive and Gram-negative bacteria, emphasizing the complexity and potential of OMW phenolics in inhibiting microbial growth.



Figure 4 Olive mill wastes

Earlier studies linked OMW toxicity to low molecular weight phenolics, particularly monomeric phenolic compounds. However, recent research has indicated that other factors also contribute to OMW acute toxicity, as reducing monomeric phenolics may not necessarily alleviate toxicity. The exact mechanism of OMW-induced toxicity remains unclear, but it is suggested that various OMW compounds, including phenolics, may exert a narcotic action on seeds and early plants through noncovalent membrane interactions. Toxicity may also arise from bioactive intermediate compounds formed during the transformation of phenolics. Additionally, OMW has been found to decrease the phosphorylation efficiency of mitochondria, likely due to structural changes induced in the inner mitochondrial membrane by organic compounds like fatty acids present in OMW. Phenolic compounds, such as p-coumaric acid and ferulic acid, can affect the physiology of both prokaryotic and eukaryotic organisms. Other OMW components, such as lipids, cannot be excluded as potential contributors to toxicity. The low pH and osmotic stress caused by high Na⁺ and Cl⁻ concentrations may also play a role in OMW acute toxicity. Microbial communities in OMW may directly influence acute toxicity, particularly against aquatic biota. Specific indigenous microbial taxa, like *Aeromonas hydrophila* and *Enterobacter cloacae*, have been linked to negative effects on the aquatic crustacean *Thamnocephalus platyurus*, highlighting the importance of assessing microbial communities in OMW for both bioremediation and safe disposal purposes. On the other hand, OMW's total phenolics content can be harnessed for controlling and inactivating plant and human pathogens. For example, hydroxytyrosol-rich OMW extract exhibited fungicidal action against the soil-borne plant pathogen *Verticillium dahliae*. Phenolic compounds from OMW and TPOMW have shown promise in inactivating pathogenic bacteria and their toxins. The phenolic substrate 4-hydroxytyrosol, when administered, can inactivate *Staphylococcus aureus* without being cytotoxic to spleen cells, concurrently reducing the biological activity of staphylococcal enterotoxin A. Furthermore, the molluscicidal activity of olive mill waste phenolics and the suppressive properties of TPOMW extracts against weeds and nematodes have also been reported.

4. Effects of Olive Mill Wastes and Olive Mill Wastes-Derived Composts on Soil Microbiota

4.1. Effects of Olive Mill Waste Spreading on Soil Microbiota

The land application of olive mill waste (OMW) appears to enhance soil microbial activity. Controlled spreading of OMW can lead to an increase in the total soil microbial population, accompanied by a rise in the abundance of spore-forming bacteria, *Actinobacteria*, and yeasts. However, the influence of OMW application on bacterial community structure is soil-dependent, with significant effects observed in loamy sand soils, possibly due to the higher availability of OMW phenolics in such soil types. Mechri et al. conducted a study using fatty acids methyl esters (FAME) analysis and found that OMW application specifically affected the actinobacterial community structure in loamy sand soils. The shifts in

microbial communities during OMW spreading are attributed to factors such as lowered oxidative conditions, the presence of phenolics, and nitrogen immobilization in the soil. Interestingly, despite the increase in spore-forming bacteria reported in some studies, a decrease in Gram-positive:Gram-negative FAME quotients was observed during successive OMW applications, suggesting a higher proportion of metabolically active Gram-negative bacteria compared to dormant spore-forming populations. The rise in total soil microbial population is further evidenced by the increased soil respiratory activity, which is linked to the degradation and assimilation of organic substrates. Long-term OMW applications have been shown to favor the relative abundance of fungi over bacteria in the soil, primarily due to the early decomposition of labile organic matter. *Actinobacteria*, *yeasts*, and certain Gram-negative taxa, like *Pseudomonas spp.*, are recognized as effective degraders of recalcitrant compounds, contributing to the breakdown of complex OMW components in the soil. However, it should be noted that long-term OMW applications can also lead to elevated total coliform counts in the soil, indicating the need for careful monitoring and management of OMW land application to ensure environmental safety.



Figure 5 Effects of olive mill waste on soil

When TPOMW (two-phase olive mill waste) is applied to soils, it results in an increase in fungal diversity while causing a decrease in bacterial diversity. Phospholipid fatty acid (PLFA) analysis in TPOMW-amended soils shows a gradual shift in the ratio of Gram-positive to Gram-negative bacteria, with a stimulation of microbial activity observed through dehydrogenase and fluorescein diacetate hydrolase assays. Extended applications of OMW can lead to an increase in the abundance of soil denitrifying communities, but the nitrifying population is suppressed due to the reducing power of OMW phenolics. Ammonia-oxidizing bacteria (AOB) are particularly affected, being highly suppressed in the presence of OMW, while members of cluster 3 of *Nitrosospira* are proliferated. As a consequence, the nitrification process is inhibited, impacting the soil nitrogen cycle. OMW applications significantly influence soil basidiomycete communities, although the effects can be alleviated with N fertilization. Changes in basidiomycete community structure are linked to the addition of organic matter and N immobilization during OMW spreading. OMW application to soils can cause transient changes in arbuscular mycorrhizal colonization of plants like *Vicia faba* by *Glomus spp.*, but the population is restored after long-term plant growth. However, high OMW dose applications (greater than 30 m³ ha⁻¹) have been reported to decrease the relative proportion of the 16:1 ω 5 FAME biomarker in olive tree rhizosphere soils, indicating a suppression of arbuscular mycorrhizal populations under such conditions. This decrease is associated with increased C/N ratio, total P, and phenolics concentrations observed after long-term OMW spread. The input of *arbuscular mycorrhizas* to plants growing in olive mill dry residue amendments depends on the type of the plant and the *arbuscular mycorrhizal* species, as indicated by Sampedro et al. in their study.

4.2. Microbial Diversity in Olive Mill Wastes-Based Composts and the Effects of Their Amendments on Soil Microbiota

Functional diversity in olive mill wastes is relatively low but increases during aerobic treatment. Composting or vermicomposting of olive mill wastes results in higher activities of dehydrogenase, β -glucosidase, and urease enzymes due to the transformation of phenolics. Composted olive mill wastes commonly exhibit high extracellular enzyme activities, particularly for esterases and ureases. Carbon and nitrogen content in olive mill waste-based composts influence the functional and catabolic diversity of indigenous microbiota. TPOMW microbiota has shown the ability to oxidize a wide range of carbon substrates. Composting or vermicomposting can shift bacterial diversity, favoring the abundance of *Alphaproteobacteria* and *Actinobacteria* over *Betaproteobacteria*. Olive mill waste-based composts support the growth of bacterial communities specialized in organic matter decomposition, particularly phenolics, tannins, and lipids. Tannase-expressing bacterial communities consisting of *Actinobacteria*, *Firmicutes*, and *Proteobacteria* have been identified in TPOMW-based composts, while various *Bacillus spp.* isolated from OMW exhibit strong lipolytic activities. Olive mill waste-based composts have shown potential benefits in the bioremediation of

contaminated soils due to the presence of microbial consortia with degradation abilities. They have been successfully used for the bioremediation of trichloroethylene and polycyclic aromatic hydrocarbons (PAHs)-contaminated soils, leading to changes in bacterial communities and enhanced enzymatic activities like naphthalene dioxygenase.

5. Microbial Community Structure in Bioreactor Systems Treating Olive Mill Wastes

Bacterial diversity during acidogenesis of OMW in anaerobic packed-bed biofilm reactors showed different community structures depending on the support material used. Granular activated carbon favored *Betaproteobacteria* and *Gammaproteobacteria*, while ceramic cubes favored *Bacillus*, *Clostridium*, *Paenibacillus*, and *Pasteuriaceae* strains. During acidogenesis of permeate in anaerobic packed-bed biofilm reactors, *Firmicutes*, particularly *Clostridium*, dominated the bacterial community. *Actinomyces* and *Staphylococcus* were minor constituents of the biofilm formed. In a granular activated carbon packed-bed anaerobic bioreactor fed with OMW, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Bacteroidetes* were identified, along with fermentative bacteria of the genera *Clostridium* and *Anaerobaculum*. *Syntrophus* spp., in syntrophic relationship with methanogenic Archaea, were detected, while sulphate-reducing bacteria were also present due to the high sulphate concentration in the OMW digested. Aerobic treatment of TPOMW-based mixtures showed an increase in the relative fungal/bacterial ratio, with predominant fungi such as *Penicillium roqueforti*, *Candida norvegica*, and *Geotrichum* sp. In nonaerated olive mill waste-derived mixtures, fungi related to *Pichia membranifaciens*, *Cladosporium herbarum*, *Ascochyta*, and *Geotrichum* were dominant. Bacterial diversity in nonaerated olive mill waste-based mixtures consisted mainly of fermentative bacteria belonging to *Firmicutes*, followed by *Actinobacteria* and *Gammaproteobacteria*. In an anaerobic CSTR treating TPOMW, bacterial diversity was dominated by *Clostridium* spp. at low organic loading rates, but consisted of *Actinobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Deferribacteres* at high organic loading rates. *Chloroflexi* spp. were also involved in the anaerobic digestion process of TPOMW. Methanogenic diversity in TPOMW-fed bioreactors under mesophilic conditions was mainly composed of *acetoclastic Methanosaeta* and *Methanosarcina* species. At thermophilic conditions, hydrogenotrophic methanogens like *Methanoculleus*, *Methanobacterium*, *Methanothermobacter*, and *acetoclastic thermophile Methanosarcina thermophila* became more dominant. In thermophilic anaerobic sludge, *Methanoculleus thermophilicus* and *Methanosarcina thermophila* prevailed. Methane production during anaerobic digestion of TPOMW in CSTRs was mainly carried out by *Methanosaeta* spp.

6. Features of Biotechnological Importance in Olive Mill Wastes Microbiota

6.1. Biodegradation of Olive Mill Wastes Using Indigenous and Selected Microbial Strains

Various microorganisms have been employed for the in vitro dephenolization and decolorization of olive mill wastes, including *basidiomycetous* and *ascomycetous* yeasts, white-rot fungi, *Aspergillus*, and *Penicillium* spp. Some of these degraders have been isolated from other environmental sources, while only a few belong to the indigenous microbiota of olive mill wastes. Certain bacterial groups, such as *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, have been found capable of degrading phenolic components of olive mill effluents through mechanisms like ring-cleavage and o-demethylation. Bacterial strains isolated from other wastewater or contaminated sites have also been used for detoxification purposes. Yeasts like *Candida*, *Geotrichum*, *Rhodotorula*, *Trichosporon*, and *Yarrowia*, as well as white-rot fungi such as *Corioliopsis*, *Ganoderma*, *Lentinula*, *Phanerochaete*, *Pleurotus*, *Poria*, and *Pycnoporus*, have been widely employed in the bioremediation of olive mill wastes. Laccases, peroxidases, and radical oxygen species are involved in the degradation process of olive mill waste phenolics in white-rot fungi. Despite using selected microbial strains for degrading recalcitrant compounds in sterile media, their effectiveness under ambient conditions may vary. Indigenous microorganisms adapted to olive mill waste conditions are more likely to be effective colonizers of the effluent. In vivo experimentation of these microorganisms is necessary to ensure their successful degradation of olive mill wastes. Additionally, genetic engineering and enzyme technology approaches may enhance the colonization and degradation potential of these microorganisms.

6.2. Bioconversion Aspects of Olive Mill Waste Microbiota

OMW microbiota has been harnessed for various biotechnological applications, including the conversion of tyrosol to phenolic compounds with high antioxidant activity. Liebgott et al. demonstrated the biotransformation of tyrosol to hydroxytyrosol and 3,4-dihydroxyphenylacetate by a *Halomonas* strain. Additionally, oleuropein, present in olive mill wastes, can also be transformed into hydroxytyrosol. Furthermore, certain phenol-tolerant Enterobacteriaceae strains isolated from OMW possess the remarkable ability to bioconvert xylose to ethanol, offering potential applications in biofuel production. Another anaerobic bacterium, *Clostridium bifermentans* TYR6, isolated from OMW, can convert cinnamic acid to 3-phenylpropionic acid. Mushroom species growing in OMW have exhibited enhanced β -glucan synthase activities, showcasing the potential for improved production of beneficial compounds. Moreover, *Paracoccus*

thiocyanatus and *Halothiobacillus neapolitanus* strains, sulfur-oxidizing bacteria isolated from alkaline TPOMW-based compost, can be utilized for compost acidification, providing a useful application in waste management and agricultural practices.

6.3. Plant Disease-Suppressive Properties of Olive Mill Wastes

Olive mill wastes (OMWs) have been found to exhibit antifungal activity against various plant pathogens. They can effectively suppress soil-borne pathogens such as *Rhizoctonia solani* and *Fusarium solani* through the antimicrobial action of OMW phenolics. Raw OMW has been reported to control *Botrytis* fruit rot on strawberries and peppers, inhibiting sporulation of *Penicillium* and *Botrytis* spp., and suppressing the phytopathogenic effects of *Fusarium oxysporum f.sp. lycopersici* on tomato plants. Bacteria isolated from OMW have also demonstrated antagonistic effects against soil-borne pathogens. Bacterial strains related to *Bacillus* and species *Burkholderia caryophylli* and *Pseudomonas fluorescens* induced disease suppressiveness against *Fusarium* and/or *Rhizoctonia* damping-off of tomato plants. Moreover, bacterial strains from OMW, including *Bacillus subtilis*, *B. pumilis*, *Pseudomonas putida*, and *Stenotrophomonas maltophilia*, exhibited in planta antimicrobial activity against *Agrobacterium tumefaciens*. In addition, *Serratia marcescens* strain BR2.1, isolated from the rhizosphere of tomato plants growing in OMW-derived compost amendments, demonstrated in planta antimicrobial activity against *Fusarium oxysporum f.sp. radicle-lycopersici*. TPOMW and TPOMW-derived compost extracts displayed general suppression against soil-borne oomycete *Phytophthora capsici*, although the effect against *Pythium ultimum* and *Botrytis cinerea* varied with the specific olive mill waste tested, and only mature compost showed protection against these plant pathogens. However, TPOMW and TPOMW-based compost extracts could not suppress *Rhizoctonia solani*. Moreover, actinobacterial strains related to *Streptomyces* and *Lechevalieria*, isolated from TPOMW-derived compost, demonstrated suppressive action against fungal and oomycete pathogens, including *Fusarium oxysporum f. sp. melonis*, *Phytophthora cinnamomi*, *Pythium debaryanum*, *Sclerotinia sclerotiorum*, and *Thanatephorus cucumeris*, as well as the bacterial strain *Agrobacterium tumefaciens* CECT 4119. The suppression of soil-borne pathogens by olive mill waste-derived compost amendments can be attributed to various factors, including competition for nutrients and ecological niches, antibiosis through the secretion of volatile metabolites or other antimicrobial agents, and inhibition of spore germination and germ tube elongation via hydrolytic enzymes. A microbial-induced suppression, associated with the dominance of copiotrophs and/or the proliferation of certain microbial groups, significantly contributes to the suppressive effects of OMW and TPOMW.

7. Conclusion

Surveillance of microbial communities is essential to comprehend bioremediation processes, yet only a few studies have been conducted to identify microbial communities in olive mill wastes (OMWs). Implementing advanced techniques like 16S rRNA gene clone libraries and high-density DNA microarray (PhyloChip) has enhanced our understanding of OMW indigenous microbiota and revealed cultivar-specific effects. However, there is a dearth of studies using high-throughput techniques, such as pyrotag sequencing and metagenomic approaches. The integration of omic approaches, like high-density 16S rRNA microarray (PhyloChip) and 16S rRNA pyrotags, alongside a comprehensive analysis of olive mill waste phenolics, physicochemical, and environmental features, can shed light on the factors influencing microbial ecology in olive mill effluents through canonical correspondence analysis. Despite the untapped biotechnological potential of olive mill wastes, novel bacterial taxa are still being identified, hinting at unexplored resources for biotechnology. Genome sequencing of indigenous microbiota will reveal the biodegradation pathways of recalcitrant compounds and their adaptive mechanisms to olive mill waste phenolics. Shifting the research focus from simple characterization to understanding the functional role of microbial communities in OMWs is crucial. This involves genome sequencing of important isolates identified in OMWs and known degraders, including uncultured ones that can be characterized through single cell genomic (SCG) approaches. Metagenomic approaches will provide insight into the genes present in OMW, while genomes sequenced will form the foundation for gene mapping, enabling a more comprehensive understanding of their functional contributions.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author's contribution

Subaranjani.S , Arunthathi. M, Danya. G and Dr.R. Mahenthiran * contributed to the paper's development.

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