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Isolation, *in vitro* evaluation and construction of Versatile Microbial Consortia

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ABSTRACT

In this study, we constructed versatile microbial consortia (VMC) with potential applications in organic waste management. After the sample and isolation procedure, the purified isolates were evaluated for their enzymatic potential, such as cellulose, xylan, petroleum, and proteins -hydrolysis. Selected isolates were screened for other traits, such as phosphate solubilization, nitrogen fixation and antimicrobial activity. Finally, the isolates were grouped into consortia according to their compatibility. The microorganisms selected for each consortium were identified by partial analysis of the 16S rRNA (bacteria) and the ITS region of the 18S RNA gene (fungi). Two microbial consortia were obtained and named VMC1 and VMC2. These two consortia are characterized by several activities of agricultural and environmental interest, such as the degradation of recalcitrant and polluting organic compounds, nitrogen fixation, IAA production phosphate solubilization and antimicrobial activity. Molecular identification of the microorganisms forming the two consortia allowed us to identify two species of actinomycetes (Streptomyces sp. BM1B and Streptomyces sp. BM2B), one species of Actinobacteria (Gordonia amicalis strain BFPx) and three fungal species (Aspergillus luppii strain 3NR, Aspergillus terreus strain BVkn and Penicillium sp. BM3). The term "Versatile Microbial Consortia" is a term that we proposed in this study to establish a methodology for building multifunctional microbial groups for the better valorization of organic waste.

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Introduction

In recent years, the application of chemical fertilizers and pesticides has increased rapidly and is a serious concern for agricultural production and environmental management. Currently, conventional agriculture and its practices are presented as a major threat to soil vitality by causing the alteration of microbial functional diversity. Intensive agriculture leads to land degradation and environmental pollution in several agroecosystems (1-3). The organic fraction of organic waste represents a precious resource that could be recycled and transformed into fertilizer rich in nutrients. Organic waste comprises recalcitrant substances such as cellulose, hemicellulose and lignin (4, 5). Soils contain a high proportion of organic matter from, for example, plant residues. This proportion is an important aspect of the function and quality of the soil (3, 6). Large quantities of organic waste from plant crops are a real environmental problem because this waste represents a real focus of microorganisms, some of which are pathogenic, which can survive in agricultural soils and infect subsequent crops (7). In addition, organic fertilizers of animal origin recycled into agricultural soils may contain pathogenic bacteria that can threaten human and environmental health (8). Also, waste polluted by hydrocarbons can affect agricultural production in terms of quantity and quality (9).

Microorganisms govern the functioning of global biogeochemical cycles (6). They have been considered an important source of natural compounds of agro-active importance (10). The soil ecosystem includes various microbial communities that perform various functions, namely, decomposition of organic matter, storage and release of nutrients for plants, regulation of plant growth, etc. (5). Bacteria dominate in the initial phases of decomposition of plant residues, while fungi predominate in later phases (3). The decomposition of high molecular weight organic matter in the soil is mediated by microbial extracellular enzymes. These enzymes are mainly hydrolases that help to acquire carbon, phosphorus and nitrogen (6, 11). The essential role of beneficial microorganisms is the decomposition of organic waste into nutritive elements, detoxification, suppression of plant diseases, improving nutrient cycling, nitrogen (N) fixation and production of many bioactive compounds, including vitamins and hormones (12). Many studies have demonstrated the beneficial effect of microorganisms on crop yields and quality. However, the use of microbial consortia in agriculture remains low. Due to the synergy of the microorganisms that populate them, microbial consortia have more properties than an individual inoculum (1). A microbial consortium is made up of several microbial species working together with broad metabolic capacities (13). The use of microbial consortia in organic waste degradation is sure, efficient and econom-

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ical (14).

In this work, the term « Versatile Microbial Consortia (VMC) » refers to a group of microorganisms that can ensure many functions simultaneously, i.e., degradation of recalcitrant compounds, nitrogen fixation, phosphate solubilization, antimicrobial activity, hydrocarbons degradation, etc. Hence, the objective of this study is to construct VMC with multiple functions that can serve potential applications in organic waste management.

Materials and Methods

Sampling and isolation

Isolation of cellulose-degrading microorganisms

To isolate cellulose-degrading microorganisms, ruminant manure and soil were sampled in sterile bags and transported to the laboratory. Then, a dilution series was prepared and 100 µL of each dilution was spread on the surface of a Petri dishes containing the mineral salt medium (MSM) composed in g/l: NaCl (0.5), $(NH_4)_2SO_4$ (0.1), $NaNO_3$ (0.2), $MgSO_4.7H_2O$ (0.025), $K_2HPO_4.3H_2O$ (1.0), KH_2PO_4 (0.4), agar (15), and pH adjusted to 7.0 (15) supplemented with 1% carboxymethylcellulose (CMC) as the sole carbon source. Petri dishes were incubated at 30°C until colonies appeared. The colonies which appear in the highest dilutions were purified on the same medium. Pure cultures were obtained by successive sub-culturing on appropriate medium and stored at 4°C for later use.

Isolation of petroleum-degrading bacteria

To isolate petroleum-degrading bacteria, 50µl of the stock sample solution (fresh sewage sludge collected from a wastewater treatment plant, Ibn Ziad-Constantine and mixed with sterile distilled water at 10%) was mixed with 200µl of the sterile crude oil, which covers the surface MSM-agar then incubated at 30°C (15). After incubation, two successive subcultures were carried out on a new surface of MSM-agar medium covered with 200µl of well-spread sterile crude oil, then incubated at 30°C. Pure cultures were obtained by successive sub-culturing on appropriate medium and stored at 4°C for later use. Bacteria isolated on MSM-crude oil agar were evaluated for degradation of crude oil in a liquid medium. Erlenmeyer flask-100ml containing 30ml of liquid MSM medium supplemented with 1% crude oil as the sole carbon source was inoculated with 200µl of bacterial suspension washed twice in PBS solution (8000rpm/10min), then incubated at 30°C under continuous agitation (150rpm) for 10 days.

In vitro detection of some potential traits of purified isolates

Purified isolates were evaluated for their enzymatic potentials, such as hydrolysis of protein (16), starch (17), cellulose (18), xylan (19), lipid (20), urea (21) and gelatin (22); production of melanin (23) and ammonia (NH_3) (24); nitrogen fixation (25). Other tests are detailed below.

Phosphate solubilization

Solubilization of phosphate (P) was evaluated on Pikovskaya medium (PVK) containing in g/L: (10), $Ca_3(PO_4)_2$ (5), $(NH_4)_2SO_4$ (0.5), NaCl (0.2), $MgSO_4.7H_2O$ (0.1), KCl (0.2), Yeast extract (0.5), $MnSO_4 \cdot H_2O$ (0.002), $FeSO_4 \cdot 7H_2O$ (0.002), Agar (15), and pH adjusted to 7. The inoculation was done, on a Petri dish containing the PVK medium, by streaking in the case of bacteria and actinomycetes and by deposit of agar disk for fungi. The appearance of a clear area around the colonies indicates the solubilization of P (26). We also studied, for fungi, the solubilization of P in test tubes containing PVK medium by agar disk deposition of each fungus. The appearance of a clear area below the fungal disk indicates the P solubilization.

Indole acetic acid (IAA) production

After each incubation period, the supernatant of bacterial cultures and fungi was collected in sterile Eppendorf-1.5ml tubes and then centrifuged (14,000 rpm, 15 min). After centrifugation, the quantification of IAA in the supernatants was estimated by the colorimetric method and detected by thin-layer chromatography (TLC) (10, 27).

Biodegradation of feather waste

Proteolytic isolates were evaluated for their potential to degrade keratin waste. For this, fresh and whole poultry feathers were washed with distilled water and then dried. Thirty milliliters of minimal medium [$MgSO_4.7H_2O$ (0.2g/l), K_2HPO_4 (0.3g/l), KH_2PO_4 (0.4g/l) and $CaCl_2$ (0.22g/l)] were introduced into each 100ml Erlenmeyer flask containing three poultry feathers and autoclaved. Erlenmeyer flasks were inoculated with each proteolytic isolate and then incubated for 21 days under continuous agitation (200rpm) (28).

Antimicrobial activity

The antagonism of the selected isolates was studied by cross-streak method (29) against four pathogenic bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076, obtained from National Center for Biotechnology Research, Constantine, Algeria). While antifungal activity was investigated against three phytopathogenic fungi: *Aspergillus niger* ATCC 9642, *Botrytis cinerea* BC1 (obtained from the mycology laboratory of Bejaia university, Algeria) and *Fusarium* sp. F6 (obtained from National Center for Biotechnology Research, Constantine, Algeria) using the direct confrontation method on the Petri dish (30). The antagonism tests were performed in duplicate

Grouping of selected isolates into a consortium

The cross-streak method was used to group the selected isolates into a consortium (14). Each isolate was streaked along the center of the Petri dish or by deposition of an agar disc in the case of fungi on TSA medium, then incubated for 24h for bacteria and 5 days for actinomycetes and fungi. After incubation, the isolates were inoculated with a streak perpendicular to the pre-incubated streaks and then incubated for 48h for bacteria and 5 days for actinomycetes and fungi. Isolates are grouped in the same consortium when no antagonism has been observed between them.

Morphological and molecular characterization of selected isolates

The isolates that make up the two consortia VMC1 and VMC2 have been morphologically characterized and identified by analysis of the rDNA gene. In the case of bacteria, DNA extraction and PCR amplification of the 16S rRNA region were performed as described by Ferrari et

al. (31) and Pudasaini et al. (32), respectively. For fungi, DNA extraction and PCR amplification of 18S rRNA IST regions were performed as described by Azevedo et al. (33). The PCR amplicons obtained were purified and sequenced (33). The obtained sequences were subjected to sequence similarity comparison using the NCBI Gene Bank database. To build the phylogenetic tree, sequences were aligned using the multiple sequence alignment program from the MEGA 11 program.

Results and Discussion

Isolation and purification

This work was carried out by isolating microbial strains from the soil, sewage sludge and ruminant manure. The purification allowed us to have 11 different isolates (Six cellulose-degrading microorganisms and five petroleum-degrading bacteria), which include fungi, actinomycetes and bacteria. The number of purified isolates on the isolation media and the isolation sample are presented in Table 1.

The evaluation of the growth of petroleum-degrading bacteria in the liquid medium allowed us to select the BFPx isolate, which showed good growth (Figure 1). Consequently, seven isolates were selected for further work. A similar approach was taken by Saha and Santra (34) to isolate microorganisms from municipal solid waste for use in organic waste degradation. In order to select petroleum-degrading bacteria, Mukred et al. (35) evaluated the growth of 62 bacterial isolates on MSM-crude oil agar and then on MSM- crude oil liquid. Only four isolates showed good growth and degradation of crude oil.

Enzymes activities and other features

Most isolates purified from different samples have a diverse and important enzyme arsenal, particularly CMCase (Isolates 5NR, BM1B and BM2B), xylanase (Isolates 5NR, BM3 and BM1B), amylase (Isolates BM1B and BVkn), protease and lipase (Isolate 5NR), and gelatinase (Isolates BM3, BM2B, BVkn and BM1B) (Table 2). Saha and Santra (34) reported a similar study. They subjected nine isolates to a qualitative test for producing eight extracellular enzymes such as cellulase, lipase, protease, amylase, lecithinase, etc. In addition, Sarkar and Chourasia (14) screened isolated bacteria to produce extracellular enzymes necessary for the effective degradation of organic solid waste. In order to screen fungal isolates for their multiple functional traits and impact on plant growth, Imran et al. (11) evaluated 73 isolates for extracellular enzyme production. They found that 95.52%, 61.11%, 35.82% and 41.79% of isolates were positive for lipase, amylase, chitinase and cellulase, respectively.

Extracellular enzymes play an important role in agriculture and the environment. They play an important role in nutrient cycling by enhancing the decomposition of residual organic matter in the soil, thereby providing nutrients to plants. However, their direct role in promoting plant growth is less explored (11, 5). Cellulase and xylanase play an important role in degrading biomass rich in cellulose and hemicelluloses (36). Microorganisms such as *Penicillium* sp., *Aspergillus* spp., *Trichoderma* sp., *Streptomyces* spp. and *Bacillus* spp. produce cellulolytic enzymes during organic waste degradation (4). Lipases were used in the pretreatment of sludge resulting from the treatment of wastewater and dairy products to reduce the fat content (37). In addition, lipolytic activity promotes the biodegradation of organic pollutants such as oil waste

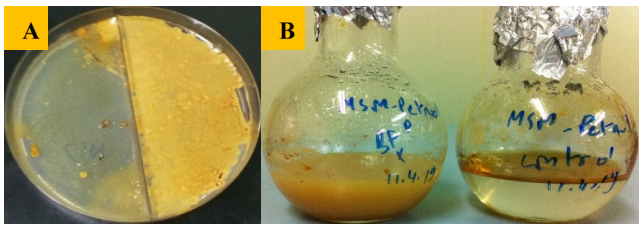

Figure 1. Growth of isolate BFPx in the crude oil-based medium: (A) on a Petri dish (right compartment) containing an MSM-crude oil agar medium, and (B) In the Erlenmeyer flask (left) containing the MSM-crude oil liquid medium.

Table 1. The purified and selected isolates from different samples.

Sample	Isolation medium	Number of purified isolates with coding	Selected microorganisms
Soil	MSM-CMC-Agar	Two isolates named 5NR and 3NR	Isolate 5NR (Actinomycetes) and isolate 3NR (Fungi)
Ruminant manure	MSM-CMC-Agar	Four isolates named BM2B, BM1B,BM3 and BVkn	Isolates BM2B and BM1B (Actinomycetes), isolates BM3 and BVkn (Fungi)
Sewage sludge	MSM-crude oil agar	Five isolates named BS_2^P, BS_1^P, BF_2^P, BFPx and BF_3^P	Isolate BFPx (Bacterium)

Table 2. Enzyme activities of selected isolates.

Isolate	Protease	Lipase	Amylase	Cellulase	Xylanase	Gelatinase
BM2B	-	+++	+++	++	-	+
BM1B	-	+	++	+++	+++	+
BM3	++	-	++	++	+++	+
BVkn	ND	-	+++	+	++	+
5NR	+++	+++	+	+++	+++	-
3NR	+	-	ND	++	++	ND
BFPx	-	-	-	-	-	ND

(+) Low activity, (++) Good activity, (+++) Excellent activity, (-) No activity, (ND) Not determined.

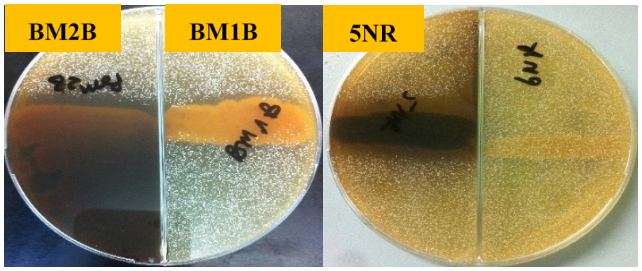

Figure 2. Melanin production by actinomycete isolates BM1B, BM2B and 5NR on a medium containing tyrosine as a precursor. Production of brownish diffusible pigmentation by isolates BM2B and 5NR.

(36). Proteases hydrolyze protein waste into amino acids and bioactive peptides. Hydrolysis products such as proline play an important role in immobilizing heavy metals by protecting plants from their toxic effect (38). Thus, protein hydrolysates derived from organic waste are generally considered plant biostimulants (39).

For melanin production only, actinomycetes isolates BM2B and 5NR produced brown to blackish pigmentation (Figure 2). Actinomycetes are known to produce melanin pigments. El-Naggar and El-Ewasy, (40) screened one hundred and thirty strains of actinomycetes for melanin synthesis and noted the significant production by *Streptomyces glaucescens* NEAE-H. The environmental interest of microorganisms producing melanoid pigments is their biological activities, including antimicrobial and antioxidant activities, as they can hunt and adsorb heavy metals such as Cd and Pb (40). Tyrosinase is the key enzyme in melanin production using L-tyrosine as a precursor (23). This precursor can be released following the action of microbial proteases on protein waste. Tyrosinase has also removed phenolic compounds from wastewater (41).

For NH_3 production, the positive isolates are BM1B, BM2B, 3NR and BM3. While for urease, the positive isolates are 5NR, BM2B, BM3 and BVkn. The study of growth in the N-Free medium showed that isolates BM2B, BM1B and BFPx are able to fix atmospheric N. Rodrigues et al. (42) estimated that 29% and 24% of isolated actinomycetes were able to grow in the nitrogen-free medium and produce ammonia, respectively. Nitrogen is one of the most important elements in molecule biosynthesis (42). Therefore, its fixation, transformation and recycling in organic and inorganic forms are of agricultural importance. For example, N values can be improved due to atmospheric N fixation by nitrogen-fixing bacteria, which usually occurs at the end of composting (43) as they can convert nitrogen gas into NH_3 in agricultural soils, which plants then take up.

Solubilization of phosphate

The selected isolates were evaluated for solubilization of inorganic P. Only fungal isolates 3NR and BM3 showed the ability to solubilize P. The study of the solubilization of P by fungal isolates 3NR and BM3 in test tubes containing the PVK agar medium allowed us to conclude on their ability to solubilize the P, while this solubilization by the same isolates on Petri dishes did not show clear solubilization (Figure 3). The use of test tubes containing PVK agar medium to reveal the solubilization of P was first described in this study. This method is mainly suitable for fungi. Phosphorus is one of the main nutrients limiting plant growth (26). Despite its abundance in soil, only 0.1% of total P exists in a form available to plants (44). Phosphate-solubilizing microorganisms ensure a soluble P form and stimulate plant growth. Doilom et al. (45) isolated phosphate-solubilizing fungi (*Aspergillus*, *Penicillium* and *Talaromyces*), of which the airborne fungal strain KUMCC 18-0196 (*Aspergillus hydei* sp. nov.) showed the most significant phosphate-solubilizing activity.

Indole acetic acid production

The production of IAA increases gradually with the incubation time. Both actinomycete isolates BM2B and BM1B showed remarkable IAA production after 12 days of incubation (33.17 and 29.1 µg/ml, respectively). In the case of fungi, the production of IAA increases progressively with the incubation time in all the fungal isolates except the 3NR isolate, which marked a decrease after the 10th day of incubation. To confirm the presence of IAA in microbial cultures, we used TLC. TLC shows pink spots relative to the distance traveled by each extract, which confirms the presence of IAA in the extract (Figure 4). In the case of the BM3 isolate, a brown-colored spot was observed, which is not at the same level as the standard. This indicates that this isolate does not produce the IAA. IAA is a common natural auxin resulting from the metabolism of L-tryptophan by microorganisms. Several microorganisms have the ability to produce IAA and improve plant growth (27). Screening of several actinomycete strains by Anwar et al. (10) for IAA production shows significant production in *Streptomyces nobilis* WA-3, *Streptomyces Kunmingenesis* WC-3 and *Streptomyces enissocaesilis* TA-3 producing 79.5, 79.23 and 69.26 µg/ml IAA. Imran et al. (11) evaluated 73 fungal isolates for IAA production. They found that 65.67% of the isolates produced IAA.

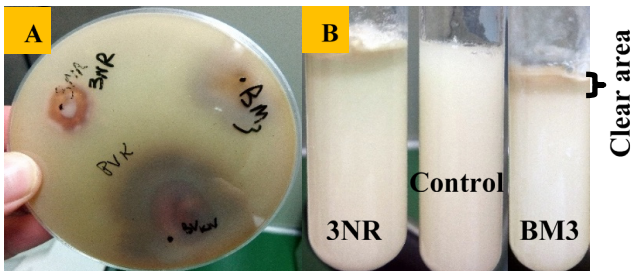

Figure 3. P-Solubilization by fungal isolates 3NR and BM3. (A) on Petri dish containing PVK medium, (B) in tubes containing the PVK medium proposed in this study, clear zone indicates P solubilization by the fungal isolates.

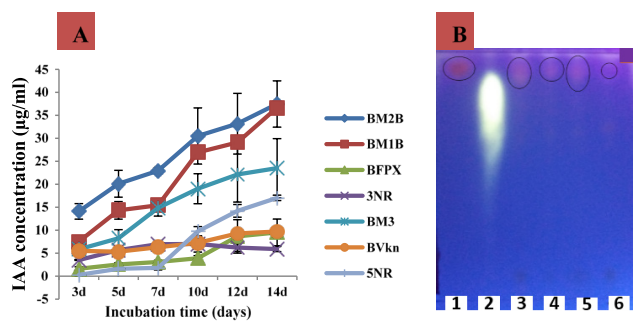

Figure 4. **(A)** IAA production as a function of incubation time by the selected isolates. (**B**) Detection of IAA by TLC, (1): Standard solution of AIA at 700µg/ml, (2): crude extract of BM3, (4): crude extract of BM2B, (5): crude extract of BM1B.

Biodegradation of feather waste

The selected isolates were evaluated for degradation of keratin waste. Isolate 5NR showed almost complete degradation of poultry feathers and partial degradation in the case of isolate BM3 after 21 days of incubation (Figure 5). Several microorganisms are responsible for the degradation of keratin-based waste. Tiquia et al. (46) isolated two keratin-degrading bacteria identified as *Streptomyces* sp. and *Bacillus licheneformis* in order to use them as an inoculum to improve the composting process. Poultry feathers have become one of the main pollutants due to their recalcitrant nature and possibly the presence of pathogenic microorganisms. They consist of 90% keratin which is a good source of peptides, amino acids and N (44, 46). The action of these microorganisms on feathers can generate peptides and amino acids of agricultural and environmental interest. The release of tryptophan during feathers degradation can serve as a precursor for the production of phytohormone (IAA) (44). Also, the hydrolyzate of poultry feather proteins obtained after bacterial degradation showed its potential in hexavalent chromium reduction (47).

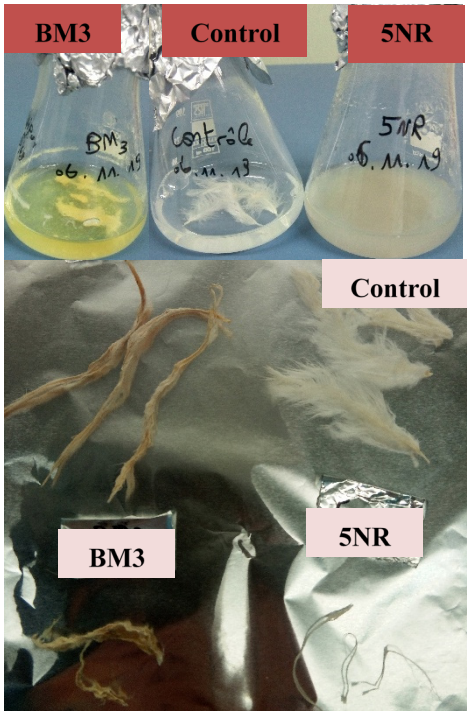

Figure 5. Degradation of keratin waste by proteolytic isolates BM3 and 5NR: (A) in minimal liquid medium supplemented with poultry feathers as a substrate, (B) appearance of poultry feathers at the end of incubation with proteolytic isolates.

Antimicrobial activity

The study of the antibacterial activity of the selected isolates showed, in general, low activity of our isolates against the pathogenic bacteria tested. Two actinomycete isolates, 5NR and BM2B, showed antagonism against *S. enteritidis* ATCC 13076 and *S. aureus* ATCC 25923 (Table 3). A similar approach was taken by Saha and Santra (34) to assess the antagonism of microbial isolates against *S. aureus*, *Salmonella* sp. and *Klebsiella pneumoniae* with the aim of using it to improve the degradation of organic waste. Actinomycetes are known for their antagonism against pathogenic bacteria. In their study, Baskaran et al. (29) screened 42 actinomycetes against pathogenic bacteria and found that 22 species exhibited antagonism. Several studies have reported the potential of some pathogenic bacteria to persist in soils amended with mature compost, such as *Salmonella* spp., *Listeria* spp. and Non-pathogenic *E. coli* (8). The presence of pathogenic bacteria in agricultural soils allows consideration of antibacterial activity as a main criterion when selecting microorganisms of agricultural interest.

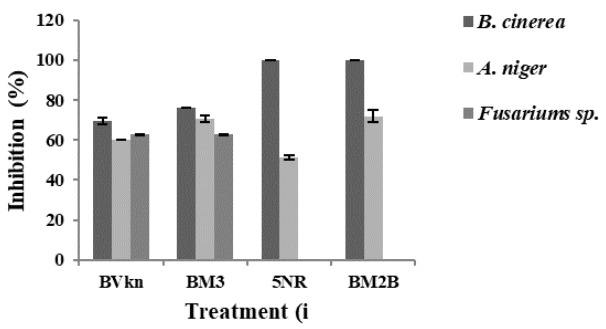

Figure 6. Percent inhibition of phytopathogenic fungi by fungal (BVkn and BM3) and actinomycetes (BM2B and 5NR) isolates obtained by direct confrontation test on Petri dishes. ANOVA, Fisher LSD test ($p \leq 0.05$).

The antagonism of the selected isolates was tested against three plant pathogens. Fungal isolates, BM3 and BVkn, have an antagonism against *B. cinerea*, *A. niger* and *Fusarium* sp. F6, while actinomycete isolates 5NR and BM2B, have an antagonism against *B. cinerea* and *A. niger* (Figure 6). Several authors claim that the development of new biological control products against plant diseases requires the screening of a large number of antagonistic agents (7). In their study, Suárez-Estrella et al. (7) screened, *in vitro*, a microbial collection for its antagonism against phytopathogenic agents and they revealed that 76 strains (25 Actinobacteria, 28 fungi and 23 bacteria) are active against *F. oxysporum* f.sp. *melonis*. To have a microbial consortium to use in composting vegetable waste, Al-Dhabi et al., (2) evaluated the antagonism of 37 actino-

Table 3. Antagonism of selected isolates against pathogenic bacteria.

Isolate	Antagonism against test bacteria			
	Gram-positive		Gram-negative	
	B. cereus	*S. aureus*	*S. enteritidis*	*E. coli*
5NR	-	+	+	-
BM2B	-	+	+++	-
BM1B	-	-	-	-
BM3	-	-	-	-
3NR	-	-	-	-
BVkn	-	-	-	-
BFPx	-	-	-	-

(+): low activity (≤9 mm); (+++): good activity (12-15 mm); (-): no activity.

mycetes against five phytopathogenic fungi (*F. xysporum*, *F. solani*, *A. niger*, *A. flavus* and *Bipolaris oryzae*). Only a single actinomycete (*Streptomyces* sp. Al-Dhabi 30) showed potent activity against all plant pathogens tested. It is recommended to select microorganisms with antagonism to at least two plant pathogens (7).

Microbial consortia

Many microorganisms have been isolated based on their ability to degrade recalcitrant materials such as lignocellulosic compounds. In many cases of degradation efficiency, microbial consortia have been shown to be superior over single strains (13). Microbial consortia have multiple applications in sustainable agriculture and the environment allowing greater absorption of nutrients and biological control of pathogens; use of organic amendments such as compost and reduction or elimination of external inputs (1, 10). In order to build versatile microbial consortia, we assessed the compatibility of selected microorganisms by the cross-streak method. The latter allowed us to propose two consortia named VMC1 and VMC2. A similar approach has been adopted by several authors (11, 34, 14).

The two consortia formed in this study present several activities of agricultural and environmental interest. VMC1 consortium is composed of actinomycetes (BM1B and BM2B) and bacteria (BFPx) with an interesting and diversified enzymatic arsenal involved in the recycling of organic matter in the soil and in organic waste management processes such as composting. This consortium can be used to remove hydrocarbons from some polluted organic waste through the isolate BFPx, which uses crude oil as the sole source of carbon. In addition, the presence of lipolytic isolates (BM2B and BM1B) can enhance hydrocarbon biodegradation. Thanks to nitrogen metabolism (nitrogen fixation, urease and NH_3 production), VMC1 can improve the fertility of agricultural soils, therefore reducing the pollution caused by the excessive use of chemical fertilizers. In addition, this consortium produces IAA, a phytohormone that promotes plant growth. VMC1 can be used in the control of pathogens: in agriculture as a biological control agent against phytopathogenic fungi and in the environment to reduce or eliminate certain pathogenic bacteria such as *salmonella* and staphylococci when managing contaminated waste such as sewage sludge. For VMC2, it is composed only of fungi (BVkn, BM3 and 3NR). This consortium has properties in common with VMC1 (interesting and diversified enzymatic arsenal, urease, production of NH_3 and IAA, biological control of plant pathogens). In addition, VMC2 can be involved in the recycling of keratin waste such as poultry feathers and in the solubilization of insoluble P in order to ensure a source of P that can be assimilated by plants. Figure 7 shows the potential interventions of VMCs to solve some major problems affecting agriculture and the environment, mainly the management of organic waste and its application in agriculture.

Morphological and molecular identification

Isolates composing VMC1 and VMC2 were identified by microscopic observation and partial gene sequencing of 16S rRNA for bacteria and 18S rRNA for fungi (Table 4). Similarities of obtained sequences with strains available in the NCBI GeneBank are presented in Table 4.

The actinomycete isolates BM1B and BM2B were affiliated with the genus *Streptomyces*. The BFPx isolate has an identity of 99.68 with the *Gordonia amicalis* strain (accession no. MT533952.1). For fungal isolates, BVkn and 3NR have 100% identity with strains *Aspergillus terreus* (accession no. MT316343.1) and *Aspergillus luppii* (accession no. KU945877.1), respectively. In contrast, the BM3 isolate was affiliated with the genus *Penicillium*. The phylogenetic analysis of the selected isolates is represented in the form of a phylogenetic tree (Figure 8).

Conclusion

The isolation and screening of microorganisms from different sites allowed us to have a diverse and interesting microbial collection composed of actinomycetes, bacteria and fungi. All isolates show an important and diversified enzymatic arsenal, mainly CMCase, xylanase, amylase, protease and lipase. The selected isolates also show other important activities, such as degradation of keratin, gelatin and crude oil; phosphate solubilization; nitrogen fixation; antimicrobial activity, etc. To reveal the solubilization of P by fungi, we recommend the use of the test tube method proposed in this study for better visualization of P, especially for fast-growing and invasive fungi. The compatibility study of the selected isolates allowed us to group them

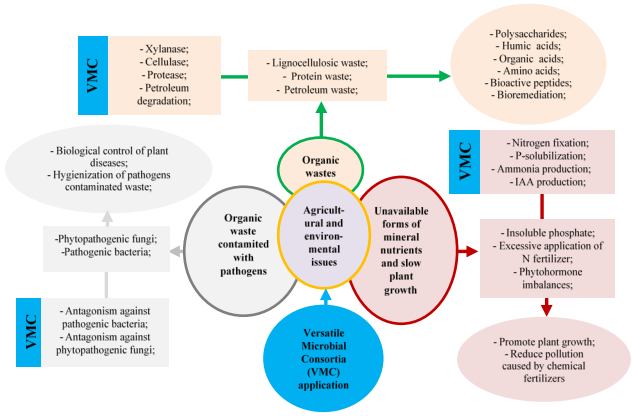

Figure 7. Potential interventions of VMC to solve some major problems affecting agriculture and the environment: potential application in organic waste management.

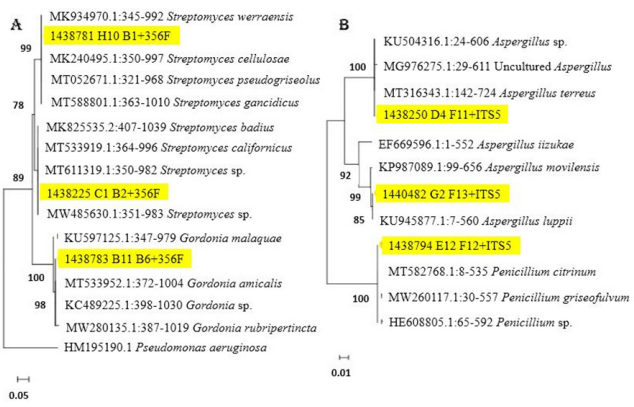

Figure 8. (**A**) Phylogenetic tree of the bacterial species composing the VMC1. The number at each branch point is the percentage supported by bootstrap in 1000. Bar, 0.05 substitutions per nucleotide position. The 16S rRNA sequence of *Pseudomonas aeruginosa* HM195190.1 was used as an out-group. (**B**) Phylogenetic tree of fungal species composing the VMC2. The number at each branch point is the percentage supported by bootstrap in 1000. Bar, 0.01 substitutions per nucleotide position.

Table 4. Identification of selected isolates based on morphological criteria as well as partial analysis of 16S and 18S rRNA. The closest relatives of the isolates, according to the BLAST search, are shown.

Strain	Microscopic appearance	Morphological identification	Next related cultivated strain (Blast)	Access Number	Percent identity (%)
BM1B (**B1**)	rectus-flexibilis spore chains	*Streptomyces* sp. BM1B	*Streptomyces gancidicus*	MT588801.1	100
			Streptomyces pseudogriseolus	MT052671.1	100
			Streptomyces cellulosae	MK240495.1	100
			Streptomyces werraensis	MK934970.1	100
BM2B (**B2**)	rectus-flexibilis spore chains	*Streptomyces* sp. BM2B	*Streptomyces* sp.	MT611319.1	99.84
			Streptomyces californicus	MT533919.1	99.84
			Streptomyces badius	MK825535.2	99.84
BFPx (**B6**)	Gram positive, rod-shaped and V-form	non-filamentous *Actinobacteria*	*Streptomyces* sp.	MW485630.1	99.68
			Gordonia amicalis	MT533952.1	99.68
			Gordonia malaquae	KU597125.1	98.74
			Gordonia rubripertincta	MW280135.1	98.43
BVkn (**F11**)	aspergillus-like conidial head	*Aspergillus* sp. BVkn	*Aspergillus terreus*	MT316343.1	100
			Aspergillus sp.	KU504316.1	100
			Uncultured *Aspergillus*	MG976275.1	99.66
BM3 (**F12**)	terminal whorl	*Penicillium* sp. BM3	*Penicillium citrinum*	MT582768.1	100
			Penicillium griseofulvum	MW260117.1	100
			Penicillium sp.	HE608805.1	100
3NR (**F13**)	aspergillus-like conidial head	*Aspergillus* sp. 3NR	*Aspergillus luppii*	KU945877.1	100
			Aspergillus movilensis	KP987089.1	99.64
			Aspergillus iizukae	EF669596.1	95.20

into two versatile microbial consortia named VMC1 and VMC2. These two consortia can fulfill several functions of agricultural and environmental interest at the same time: (i) Degradation of various organic compounds such as cellulose, hemicellulose, starch, proteins, lipids and crude oil (Potential application in the management of organic waste and the depollution of sites polluted by hydrocarbons), (ii) Phosphate solubilization, nitrogen fixation, ammonia and IAA production (potential application as a biofertilizer in agriculture) and (iii) antagonisms against pathogens (potential application in the biological control of phytopathogenic fungi and as a means of controlling pathogenic bacteria in contaminated waste). This work presents a non-exhaustive experimental methodology to follow in order to build multifunctional microbial groups for large and effective applications. However, these consortia require in situ evaluation in the management of organic waste, such as the co-composting of sewage sludge with other wastes in order to confirm their effectiveness.

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Interest conflict
The authors declare that they have no conflicts of interest.

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