biology and life sciences forum

MDPI

Proceedings

Isolation and characterization of agricultural soil bacteria with biotechnological and biological control potential applications†

Beatriz Meza-Manzaneque [1], Marta Pérez-Díaz [1], Elena G. Biosca [2] and Belén Álvarez [1,*]

[1] Departamento de Investigación Aplicada y Extensión Agraria, Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), 28805 Alcalá de Henares, Madrid, Spain; beatriz.meza@madrid.org; martap_25@hotmail.com; mariabelen.alvarez@madrid.org

[2] Departamento de Microbiología y Ecología, Universitat de València (UV), 46100 Burjassot, Valencia, Spain; elena.biosca@uv.es

* Correspondence: mariabelen.alvarez@madrid.org

† Presented at The 2nd International Electronic Conference on Microbiology, Online, 1-15 December 2023.

Abstract: Unsustainable agricultural practices eventually have an impact on soil conditions and the microbiological diversity. To regain balance, ecologically sound strategies can be an alternative. In this work, a collection of bacteria was isolated from agricultural soil and characterized to evaluate their capacity for phosphorus and iron biofertilization, exoenzyme production, and biocontrol of several phytopathogenic fungi. Bacterial identification pointed out to a majority of *Bacillus* spp. along with other several minority genera. Isolates globally displayed a high proportion of the biological activities tested, especially concerning production of hydrolytic enzymes. Inhibition on fungal growth was variable among the soil bacterial isolates by production of diffusible compounds and/or VOCs (volatile organic compounds). Evidence from this work provides promise for the application of soil bacteria to improve agricultural soil management and crop production.

Keywords: agrosystem; agrochemical; biological activity; sustainability; biofertilization; exoenzyme production; plant pathogen; biocontrol; diffusible compounds; volatile organic compounds

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Published: date

1. Introduction

Agricultural activities have a direct influence in a range of fundamental areas such as the environment, the public health, and the global economy [1]. Since many years, benefits have been achieved through unsustainable agricultural practices, among which the indiscriminate use of chemical compounds as fertilizers, pesticides and/or herbicides in the field, with the aim to increase crop yield and face an increasing demand for worldwide production. However, these conventional practices eventually have a significant impact, mainly on soil conditions and food quality [2]. The continued application of agrochemicals causes pollution in the environment. Their degradation produces chemical residues, which can remain in the field for a long time [1,2]. Treatments with fertilizers derived from nitrogen result in increased amount of nitrates in the field and disturbance of the natural nitrogen fixation process [3]. Treatments with pesticides and/or herbicides for pest chemical control favour the appearance of resistances to these products, as well as new opportunistic pests due to removal of competitors. Moreover, the global climate change has an additional effect, since it alters the distribution of crops, weeds, pests and diseases, and then, agrochemical use [1].

To regain balance and improve the efficient use of the natural resources, ecologically sound strategies as those microorganism-based can be an alternative. The beneficial interactions and activity of plant and soil microorganisms can be considered to improve agricultural development, crop production and environmental sustainability [4].

In this work, a collection of bacteria was isolated from agricultural soil and characterized to evaluate their properties for biotechnological and/or biocontrol applications, mainly capacity for phosphorus, nitrogen and iron biofertilization, exoenzyme production, and biological control of several species of phytopathogenic fungi.

2. Materials and Methods

2.1. Bacterial isolation

Agricultural soil was mixed with phosphate buffered saline (PBS) buffer [pH 7.4; 137 mM NaCl, 10 mM Na_2HPO_4, 2.7 mM KCl, 1.9 mM KH_2PO_4] at 1:10 (w/v), shaken at 200 rpm for 30 min to isolate bacteria by serial ten-fold dilutions, and plated onto Nutrient Agar (NA). Isolates were purified, PCR-identified by 27F/1492R and 27F/1525R primer sets [5], and cryopreserved. To perform the tests, they were grown on NA for 24 h at 25°C.

2.2. Biofertilization activity and exoenzyme production tests

Assays were performed according to [6,7].

Phosphate solubilization: growth onto the Pikovskayas medium (PVK) [yeast extract 0.5 g/l, dextrose 10 g/l, phosphate calcium 5 g/l, ammonium sulfate 0.5 g/l, potassium chloride 0.2 g/l, magnesium sulfate 0.1 g/l, manganese sulfate 0.0001 g/l, iron sulfate 0.0001 g/l, agar 15 g/l].

Siderophore production: growth onto the CAS medium [100 ml MM9 salt solution/750 ml ddH_2O, 32.24 g piperazine-N,N′-bis-(2-ethanesulfonic acid) PIPES, 15 g agar; after autoclaving: 30 ml Casamino acid solution, 10 ml 20% glucose solution, 100 ml Blue Dye solution]. Blue Dye [Solution 1: 0.06 g CAS/50 ml ddH_2O, Solution 2: 0.0027 g $FeCl_3$-$6H_2O$/10 ml 10 mM HCl, Solution 3: 0.073 g HDTMA/40 ml ddH_2O; Solution 1 + 9 ml Solution 2 + Solution 3].

Proteolytic activity: growth onto Skim milk agar [50 g/l milk powder, 15 g/l agar].

Gelatinase activity: growth onto Gelatin medium [0.25% yeast extract, 0.5% Bacto Peptone, 0.5% glucose, 0.1% $MgSO_4.7H_2O$, 12% gelatin].

Lipolytic activity: growth onto Tween 20 and Tween 80 media [peptone 1%, $CaCl_2.2H_2O$ 0.01%, agar 2%, Tween 20 or Tween 80 1%].

Amylolytic activity: growth onto Starch agar [potato starch 10 g/l, KNO_3 0.5 g/l, K_2HPO_4 1 g/l, $MgSO_4.7H_2O$ 0.2 g/l, $CaCl_2$ 0.1 g/l, $FeCl_3$ traces, agar 15 g/l], and addition of Lugol to the plates for 1 min.

DNase activity: growth onto DNase agar [tryptose 20 g/l, deoxyribonucleic acid 2 g/l, sodium chloride 5 g/l, agar 12 g/l], and flooding with 1N HCl for a few min.

Plates were incubated at 25 °C for all media, and the activities were monitored after 24 h, 48 h and/or 72 h, according to the test.

2.3. Biological control tests

Diffusible compound production: the soil bacterial isolates and phytopathogenic fungi of reference were cocultured onto Potato Dextrose Agar (PDA) in dual culture plate assays [8].

Volatile organic compound (VOC) production: the soil bacterial isolates and phytopathogenic fungi of reference were cocultured onto PDA in plates divided into two sections to prevent physical contact between them [9]. Plates were subsequently sealed to allow the VOCs accumulate in the inside during the experimental period.

Plates were incubated at 25 °C for both types of assays, with daily monitoring of the cocultures. Tests were performed in triplicate for each bacterial isolate.

3. Results

3.1. Bacterial identification

Molecular identification by partial *16S rRNA* gene amplification pointed out to a majority of species of Gram-positive bacteria, mainly belonging to the genus *Bacillus*, specifically to Group I, along with species from other several minority genera, as *Brevibacterium* spp. and *Enterococcus* spp., which globally accounted for more than 75 % of the total of bacterial species isolated from agricultural soil.

3.2. Biofertilization activity and exoenzyme production tests

Isolates globally displayed a high proportion of the biological activities tested, especially concerning production of hydrolytic enzymes such as proteases, lipases, amylases, gelatinases and DNases but, also production of siderophores, and other activities like solubilization of phosphates (Table 1).

Table 1. Potential of the tested bacterial isolates for biotechnological applications.

Biofertilization tests	***Bacillus* spp.**	***Brevibacterium* spp.**	***Enterococcus* spp.**	**Other genera**	**Global isolates (%)**
Phosphate solubilization	not detected	not detected	detected	detected/not detected	15
Siderophore production	detected	detected	detected	detected/not detected	49
Exoenzyme production tests					
Proteolytic activity	detected	detected	detected	detected/not detected	70
Gelatinase activity	detected	detected	detected	detected	
Lipolytic activity (on Tween 20)	detected	not detected	not detected	detected	45
Lipolytic activity (on Tween 80)	detected	not detected	not detected	detected/not detected	
Amylolytic activity	detected	not detected	not detected	not detected	23
DNase activity	detected	detected	not detected	detected/not detected	24

3.3. Biological control tests

Inhibition on fungal growth was also displayed among the soil bacterial isolates by production of diffusible compounds and/or VOCs (Figure 1) against the phytopathogenic *Verticillium dahliae*, *Fusarium pseudograminearum*, *F. oxysporum*, *Neofusicoccum parvum* and *Diplodia seriata*. Diffusible compounds were produced against the five fungal species ranging 15-30 of the isolates. Two of the isolates inhibiting *V. dahliae* also produced VOCs against this pathogen. Inhibitory effect against both *V. dahliae* and *F. pseudograminearum* was obtained with three of the isolates, against *V. dahliae* and *F. oxysporum* with three of the isolates, and against *F. pseudograminearum* and *F. oxysporum* with four of the isolates. Scarce production of VOCs was observed against *N. parvum* and *D. seriata*.

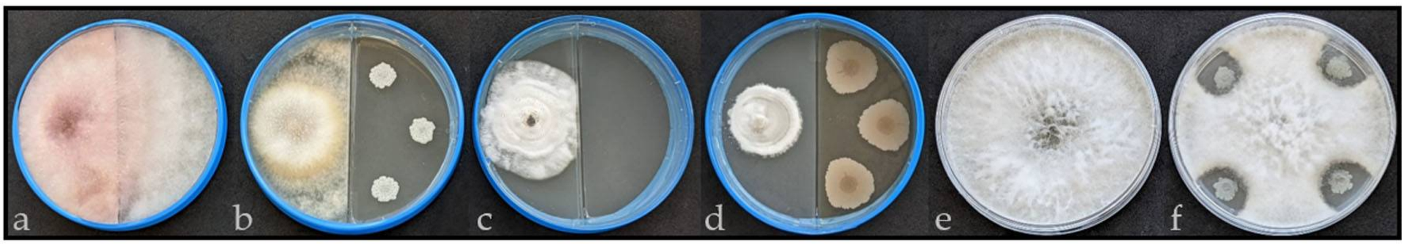

Figure 1. Potential of the tested bacterial isolates for biological control applications. Representative pictures of VOC production assays in: (**a,b,c,d**) against *Fusarium pseudograminearum* (**a,b**) and *Verticillium dahliae* (**c,d**); (**a,c**) fungal control without bacteria, and (**b,d**) bacterial inhibition. Representative pictures of a diffusible compound assay in: (**e,f**) against *Neofusicoccum parvum*; (**e**) fungal control, and (**f**) bacterial inhibition.

4. Discussion

The activities observed in the soil bacterial isolates indicate their potential to be developed and used as beneficial bacteria to contribute to improvement of soil quality and reduction in the application of agrochemicals such as fertilizers and/or pesticides to reach sustainable environmental agrosystems, similarly to observed in previous works [6,7]. Biofertilizers based on microorganisms can naturally provide crops with nutrients, mainly related to phosphorous, nitrogen, or even in smaller quantities to iron, by either increasing their efficient uptake or their availability [3]. Large-scale production in bioreactors of the exoenzymes tested could be of interest in agrifood industry. Antagonism against a pathogen can be a treatment to be included in integrated pest management programmes.

Evidence from this work provides promise for the application of soil microbiome to attain sustainable agriculture, as proposed elsewhere [10].

Author Contributions: Conceptualization, E.G.B. and B.Á.; methodology, B.M.M., M.P.D., E.G.B. and B.Á.; analysis, B.M.M., M.P.D., E.G.B. and B.Á.; resources, E.G.B. and B.Á.; writing, E.G.B. and B.Á.; funding acquisition, B.Á. All authors have read and agreed to the published version.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: B.M.M. was the recipient of a Training Fellowship by IMIDRA. Authors thank funding from IMIDRA and BACPLANT Group from Universitat de València (UV).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tudi, M.; Daniel Ruan, H.; Wang, L.; Lyu, J.; Sadler, R.; Connell, D.; Chu, C.; Phung, D.T. Agriculture Development, Pesticide Application and Its Impact on the Environment. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1112; doi:10.3390/ijerph18031112.
2. Meena, R.S.; Kumar, S.; Datta, R.; Lal, R.; Vijayakumar, V.; Brtnicky, M.; Sharma, M.P.; Yadav, G.S.; Jhariya, M.K.; Jangir, C.K.; Pathan, S.I.; Dokulilova, T.; Pecina, V.; Marfo, T.D. Impact of Agrochemicals on Soil Microbiota and Management: A Review. *Land* **2020**, *9*, 34; doi:10.3390/land9020034.
3. Thomas, L.; Singh, I. Microbial Biofertilizers: Types and Applications. In *Biofertilizers for Sustainable Agriculture and Environment*, Giri, B.; Prasad, R.; Wu, Q.S.; Varma, A., Eds.; Springer: Switzerland, 2019; Volume 55, pp. 1-20.
4. Umesha, S.; Singh, P.K.; Singh, R.P. Microbial Biotechnology and Sustainable Agriculture. In *Biotechnology for Sustainable Agriculture. Emerging Approaches and Strategies.* Elsevier, 2018; pp. 185-205.
5. Maiwald, M. Broad-Range PCR for Detection and Identification of Bacteria. In *Molecular Microbiology: Diagnostic Principles and Practice*, Persing, D.H.; Tenover, F.C.; Tang, Y.W.; Nolte, F.S.; Hayden, R.T.; van Belkum, A., Eds.; American Society for Microbiology: Washintong DC, 2011; pp. 491-505.
6. Pérez-Díaz, M.; Biosca, E.G.; Álvarez B. Looking for Beneficial Applications of Environmental Bacteria from an Olive Grove for a Microbe-Based Sustainable Agriculture. In *Global Progress in Applied Microbiology: a Multidisciplinary Approach*, Méndez-Vilas, A. Ed.; Formatex Research Center: Badajoz, Spain, 2018; pp. 64-68.
7. Esteban-Herrero, G.; Álvarez, B.; Santander, R.D.; Biosca, E.G. Screening for Novel Beneficial Environmental Bacteria for an Antagonism-Based *Erwinia amylovora* Biological Control. *Microorganisms* **2023**, *11*, 1795, doi:/10.3390/microorganisms11071795.
8. Yuan, H.; Shi, B.; Wang, L.; Huang, T.; Zhou, Z.; Hou, H.; Tu, H. Isolation and Characterization of *Bacillus velezensis* Strain P2-1 for Biocontrol of Apple Postharvest Decay Caused by *Botryosphaeria dothidea*. *Front. Microbiol.* **2022**, *12*, 808938; doi: 10.3389/fmicb.2021.808938.
9. Tahir, H.A.S.; Gu, Q.; Wu, H.; Raza, W.; Safdar, A.; Huang, Z.; Rajer, F.U.; Gao, X. Effect of Volatile Compounds Produced by *Ralstonia solanacearum* on Plant Growth Promoting and Systemic Resistance Inducing Potential of *Bacillus* Volatiles. *BMC Plant Biol.* **2017**, *17(1)*, 133, doi: 10.1186/s12870-017-1083-6.
10. Suman, J.; Rakshit, A.; Ogireddy, S.D.; Singh, S.; Gupta, C.; Chandrakala, J. Microbiome as a Key Player in Sustainable Agriculture and Human Health. *Front. Soil Sci.* **2022**, *2*, 821589, doi: 10.3389/fsoil.2022.821589.