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Pseudomonas fluorescens: Prospective green antimicrobial for crops cultivation

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ABSTRACT

Background: Throughout the 20th century, pesticides were ubiquitous in agriculture and food production as a method of controlling pest populations and maintaining high yields of crops. However, after decades of uncontrolled pesticide use, their hazardous properties were discovered by scientists. Thus, in the 21st century, the urgency of discovering a form of ecologically safe functional food production has become evident, as it is closely linked to the development of green agriculture. As a result, recent innovations in biological protection of crops have become highly relevant. In this regard, bioactive compounds produced by non-pathogenic *Pseudomonas fluorescens*, found in soil, may become a prospective bio-control agent of complex influence with potential in the targeted functionalization of crops.

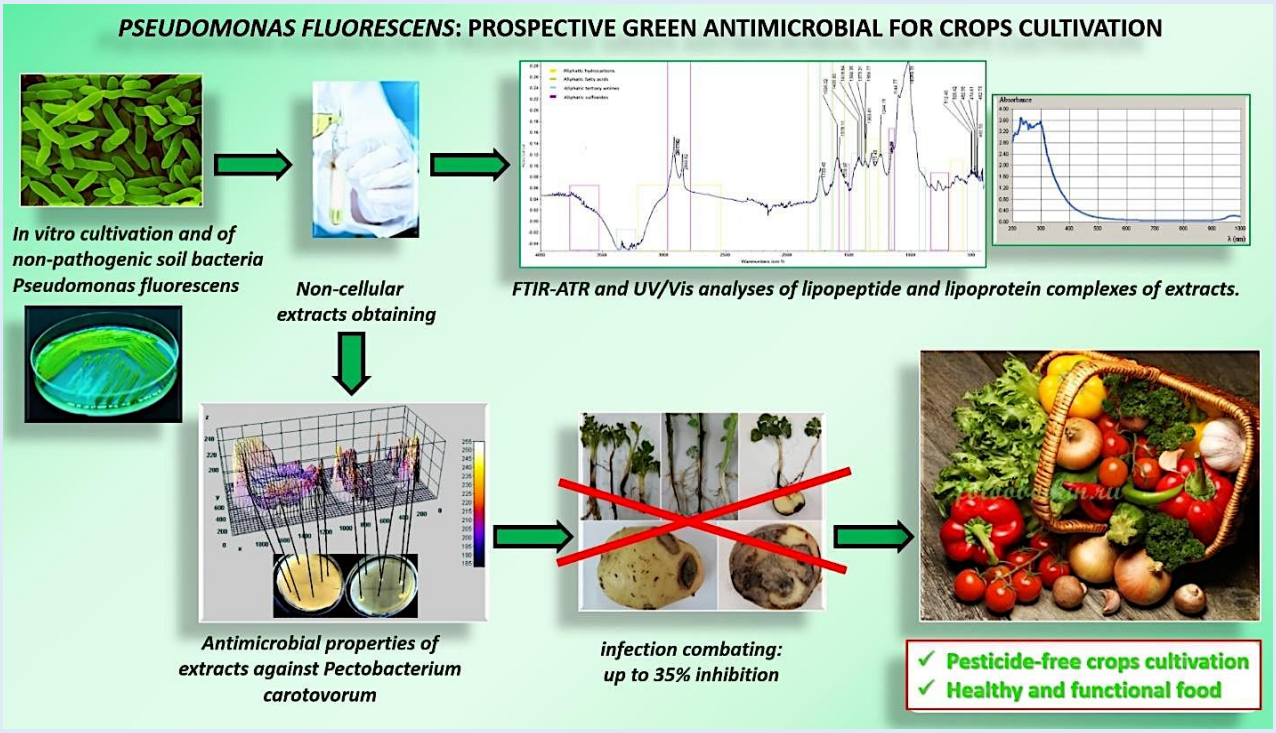
Objectives: Current research is devoted to investigation of the effect of *P. fluorescens* against the *Pectobacterium carotovorum* phytopathogen, which significantly reduces the yields of crops. Non-cellular extracts obtained from certain bacteria have been successfully tested against multi-drug-resistant strains of *P. carotovorum*.

Results: Bactericidal and bacteriostatic activities of lipoprotein and lipopeptide complexes within non-cellular extracts of *P. fluorescens* were detected. The majority of the studied strains were antibiotic-resistant and produced extracellular

proteases, lipases, and polyphenol oxidases (PPOs). Proteases and PPOs are encoded by the bacterial chromosome, while lipases can be plasmid-encoded. They are associated with the resistance and biodegradation in both bacteria but are not transmissible.

Conclusions: Within *P. fluorescens* samples, 20 strains exhibit antimicrobial activity and contain a wide variety of extracellular enzymes. According to the literature data, the presence of *P. fluorescens* in environment can enhance the antioxidant status of fruits, stimulate plant growth, boost their immunity and enhance stress tolerance. Based on all of this, *P. fluorescens* may be considered as a prospective complex biocontrol agent with potential in the functionalization of fruits and vegetables. Further research is recommended to investigate its potential as a green alternative for pesticide-free crop cultivation and soil remediation.

Key words: *Pseudomonas fluorescens, Pectobacterium carotovorum,* phytopathogen, crops biological protection, pesticide-free cultivation, food functionalization



INTRODUCTION

The 20th century has shown the rise of large-scale pesticide production and their uncontrolled use. Dangerous side effects of pesticides, including genotoxic, teratogenic, and carcinogenic impacts, were identified in the 21st century [1-2]. In the modern day, high-quality food production is directly linked to green agriculture. The problem of food safety is crucial for the sustainable development of any country, because it is also related to healthcare [3-4].

Alongside the effects of global climate change and other abiotic factors, phytopathogens and phytophagous pests pose the greatest threat to crop cultivation [5]. The main damage to crops and trees is caused by phytopathogenic bacteria, molds, parasitic fungi, and phytophagous worm pests. These include specific

microflora (tropical *Pseudocercospora punicae*, arctic *Typhula borealis* and *Pythium polare,* etc.) and common species (*Pectobacterium carotovorum, Pseudomonas syringae*, etc.). Due to both globalization and climate change, the plant protection problem has become increasingly severe [6]. Additionally, antimicrobial resistance (AMR) in pests is on the rise [7]. Therefore, the search for alternative solutions is crucial. Various biological methods of plant protection based on intraspecies interactions have been successfully implemented in the EU, UK, USA, and other countries [8-10]. In the aforementioned countries, pesticide-free cultivation of crops with the application of mentioned methods promotes the increase in food quality and in human health in general. Thus, the promotion of healthy and functional food is critical [11-13].

P. fluorescens is a common, highly adaptable, predominantly non-pathogenic soil microbe. It forms various interactions with soil microflora and produces a wide variety of enzymes and other bioactive compounds that positively affect plant root growth, increase green biomass etc. Also, the presence of *P. fluorescence* in soil increases the consistency of bioflavonoids and ascorbic acid, which increases the antioxidant status of fruits and generally improves their nutrient characteristics [14-16].

In the present study, the antimicrobial potential of *P. fluorescens* against *P. carotovorum* phytopathogen was investigated in the context of its potential application for the production of healthy and potentially functional foods. The main novelty of the present study lies in the primary screening of some local Armenian strains of *P. fluorescens* tested against the local *P. carotovorum* strains, isolated from the various regions of RA, which have not been previously studied in this context.

MATERIALS AND METHODS

Cultivation of microbial cultures: The strains from National Culture Collection of the Microbial Depository Center (MDC), "Armbiotechnology" SPC NAS RA, were studied. They were cultivated on L-agar and L-broth media at 30 °C [17]. Qualitative AMR tests were performed *in vitro* against 14 antibiotics: chloramphenicol (Cam), kanamycin (Kan), gentamicin (Gnc), streptomycin (Stp), tetracycline (Tcn), amoxiclav (Amc), amoxicillin (Amx), ampicillin (Amp), penicillin (Pcn), cefixime (Cfx), ceftriaxone (Cro), ciprofloxacin (Cip), levofloxacin (Lfx), and azithromycin (Azm), using the disk diffusion method [18].

Genetic and biochemical studies of bacterial strains: Genetic studies were performed using PCR with *catB7*, *blaOXA-10*, *aac(6')II*, *aph(3')IV* primers. Plasmids were studied using Mandel's transformation method and agarose gel electrophoresis with Safe-Green dye [19,20]. Biochemical assays were performed *in vitro* according to standard protocols for qualitative assessment of extracellular proteases, lipases, and PPOs. Tannin, α-naphthol, and L-Tyr (tyrosine) were used for PPOs detection. Polysorbates were used for lipases. Caseinase and gelatinase were assessed using milk agar and the photographic films [21-24].

***In vitro* study of interaction between *P. fluorescens* and *P. carotovorum*:** The effect of *P. fluorescens* on *P. carotovorum* growth was studied *in vitro* using classical microbiological methods. Disc diffusion method was used to assess the activity of non-cellular extracts obtained by filtering overnight cultures of each strain through nylon membranes (0.2 mcm). The bilayer agar method was applied to assess the activity of fresh cultures [25-27]. Spectrophotometry was used for experiments in liquid media [28-30]. The active components of the extracts were analyzed using UV/Vis (ultraviolet–visible) spectroscopy and FTIR-ATR (Fourier-transform infrared spectroscopy with attenuated total reflectance) [31,32].

Statistical assessment: The entire study was conducted in triplicate, with each experiment repeated three times under identical conditions. For statistical analysis, Microsoft Excel software was used. Image digitization and quantification were performed using both ImageJ and ImageJ2 software [33-34].

RESULTS AND DISCUSSION

As the first step of our research, a primary evaluation of the antimicrobial activity of *P. fluorescens* was carried out. The anti-phytopathogenic properties of *P. fluorescens* were assessed against ten *P. carotovorum* cultures. The results are presented in Fig. 1 and Table 1.

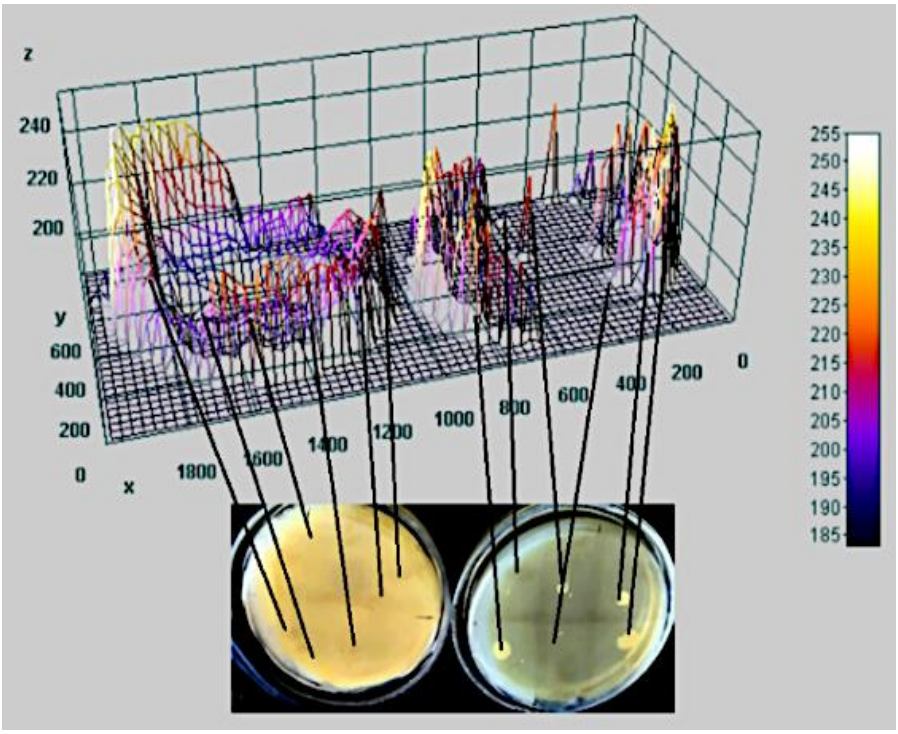


Fig. 1. Thermogram of *P. fluorescens* bacteriostatic (left) and bactericide (right) effects.

Table 1. Antiphytopathogenic effect of *P. fluorescens* against *P. carotovorum.*

P. carotovorum	*P. fluorescens non-cellular extracts*											*C*
Strain	*9068*	*9069*	*9072*	*9077*	*9085*	*9089*	*9090*	*9091*	*9092*	*9094*	*9138*	
8690	8	-	-	-	-	-	6	-	-	5	-	+
8694	9	8	10	-	-	8	8	6	-	11	-	+
8698	7	9	10	6	7	13	8	11	7	-	7	+
8702	10	-	11	11	12	8	9	11	7	9	+	+
8705	9	9	9	8	8	7	9	8	8	7	10	+
8717	8	-	7	8	8	4	6	6	7	6	-	+
8756	8	-	9	-	9	4	7	6	+	8	-	+
8764	8	-	6	-	7	6	+	7	7	6	10	+
8765	9	9	11	7	11	8	10	7	8	10	7	+
8758	9	8	10	7	8	8	8	7	8	8	9	+

Then for the understanding of the mechanisms of the discovered antimicrobial activity, the spectroscopic analyses of bioactive compounds of extracts, obtained from the most active strains of *P. fluorescens* were performed. The results of the experiments with FTIR-ATR and UV/Vis spectroscopy are presented on Fig. 2

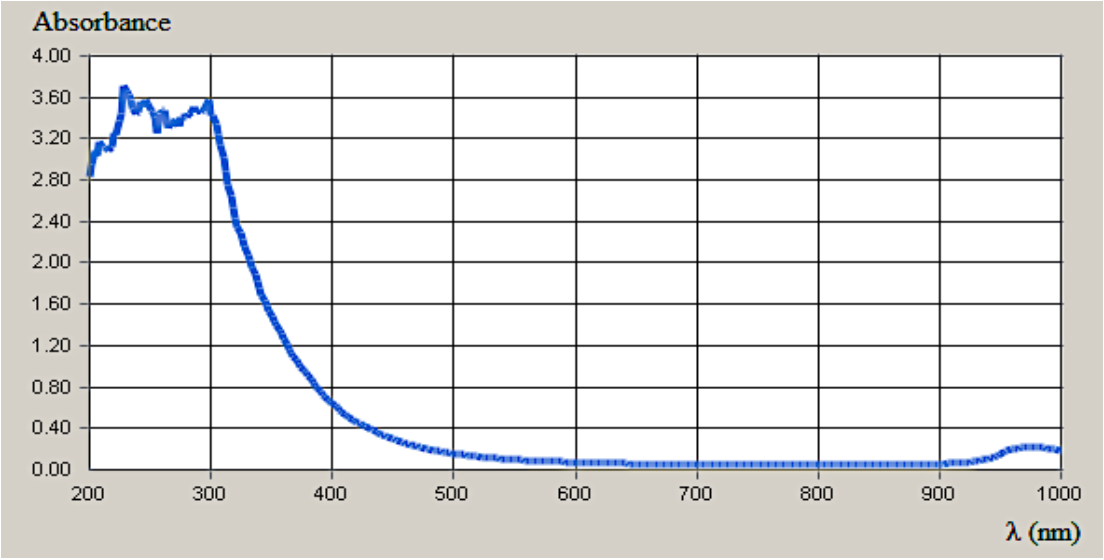


Fig. 2. UV/Vis spectroscopic study of active bactericide components of non-cellular *extract* of *P. fluorescens 9089.*

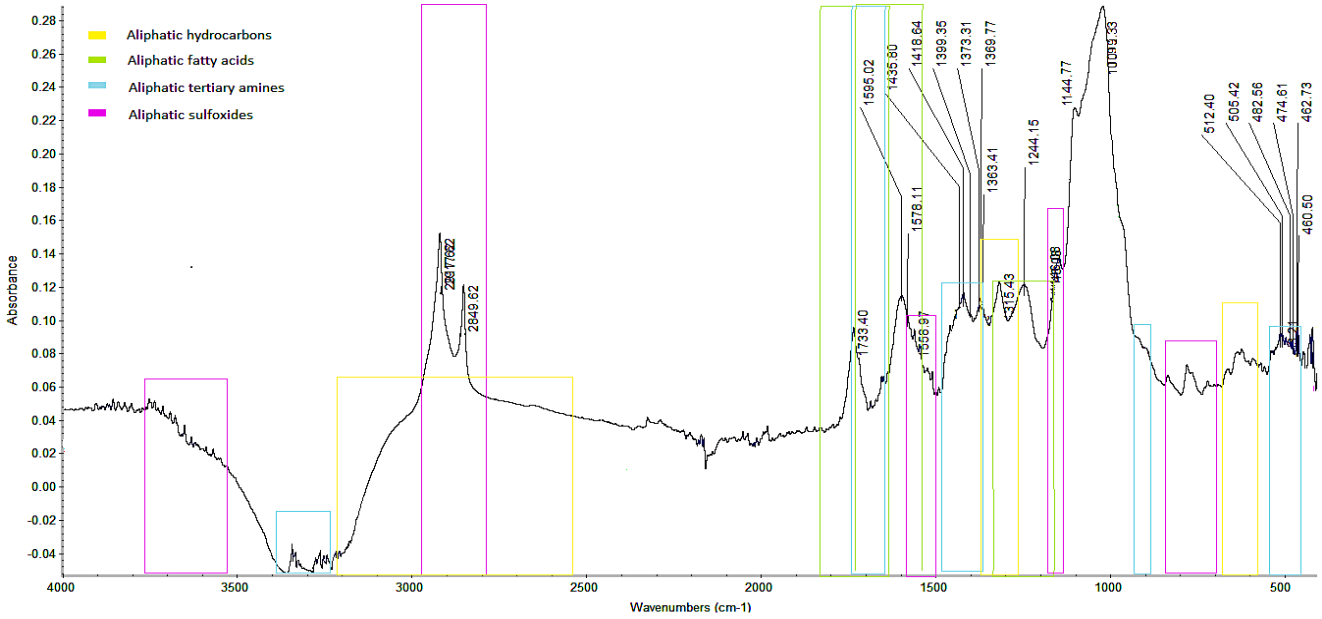


Fig. 3. FTIR-ATR spectroscopic study of active bactericide components of non-cellular extract *P. fluorescens 9089*.

Additionally, some bacteriostatic effects were observed for *P. fluorescens 9077, 9085, 9138*, and *9068*, while the remaining strains exhibited bactericidal activity. Then, AMR tests were performed, along with assessments of biodegradation and soil remediation potential (Tab. 2, Fig. 4).

Table 2. Antibiotic-resistance profiles of *P. fluorescens* typical representatives.

Strain	**Antibiotic-resistance (50 mcg/mL)**														
	Azm	Stp	Gnc	Kan	Amc	Amx	Amp	Pcn	Cip	Lfx	Tcn	Cfx	Cro	Cam	**C**
9068	-	-	-	-	-	-	R	-	-	-	R	-	-	-	+
9069	-	-	-	-	R	R	R	R	-	-	R	R	S	R	+
9077	-	-	-	-	R	R	R	R	-	-	R	R	R	-	+

Strain	Antibiotic-resistance (50 mcg/mL)														C
	Azm	Stp	Gnc	Kan	Amc	Amx	Amp	Pcn	Cip	Lfx	Tcn	Cfx	Cro	Cam	
9072	-	R	-	-	-	R	R	R	-	-	-	R	R	R	+
9085	R	-	-	-	R	R	R	R	-	-	R	R	-	-	+
9089	R	-	-	-	R	R	R	R	-	-	R	R	R	-	+
9090	R	-	-	-	R	R	R	R	-	-	-	R	R	-	+
9091	-	R	-	-	R	R	R	R	-	-	-	-	-	R	+
9092	-	-	-	-	R	R	R	R	-	-	-	R	-	R	+
9094	-	-	-	-	R	R	R	R	-	-	R	R	-	-	+
9138	-	R	-	R	-	-	R	R	R	R	R	R	R	-	+

After the series of microbiological experiments to assess antimicrobial resistance (AMR), appropriate biochemical and genetic analyses were conducted with the purpose of understanding the mechanisms underlying the detected resistance profiles of the studied bacterial cultures (Fig. 3).

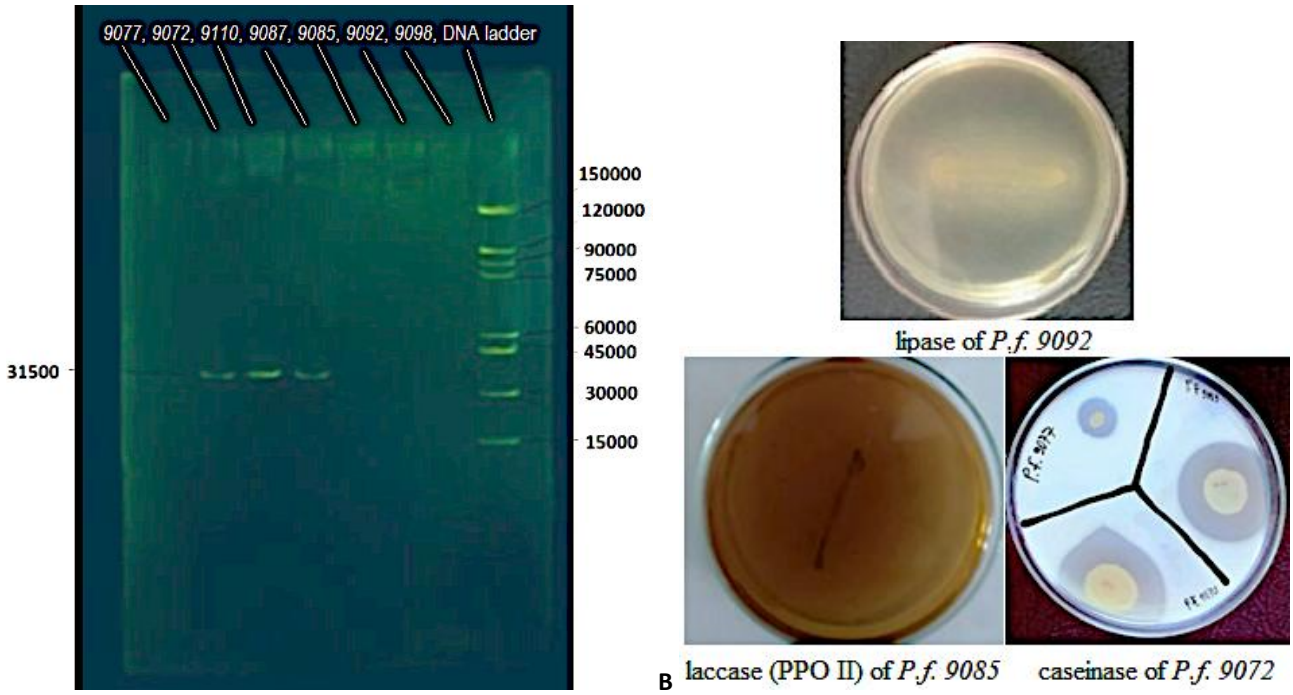


Fig. 4. PCR analysis (A) and enzyme analyses (B) of various strains of *P. fluorescens*.

The majority of the *P. fluorescens* strains exhibited AMR, indicating their high adaptive potential. Notably, both fresh cultures and non-cellular extracts of *P. fluorescens* 20 strains inhibited multi-drug resistant strains of *P. carotovorum*. *P. fluorescens* strain 9085 was maximally active. FTIR-ATR and UV/Vis analyses have shown the presence of lipoprotein and lipopeptide compounds in the extracts. The biosynthesis of analogous antimicrobial compounds (bacteriocins, keanumycins A–C, etc.) were identified in other bacterial species worldwide [35-36].

Thus, these findings underscore the relevance of investigation of antimicrobial potential of strains from the Armenian National Collection of Cultures. This research is essential for the prospective development of cost-effective and efficient biopreparations targeting phytopathogens, based on local Armenian strains.

Genetic and biochemical analyses have revealed the presence of plasmid-encoded lipases, nucleoid-associated PPOs, caseinase, and gelatinase. They are associated with biodegradative capacities and AMR of

both species [37]. The identified plasmids were not transmitted to *P. carotovorum*, due to differences in their replication control, which minimizes potential risks for their native microbiome. Furthermore, residual and trace concentrations of antibiotics, such as tetracycline, and other contaminants in soil can be biodegraded by these enzymes [38]. The application of *P. fluorescens* might be suitable for soil remediation and facilitate its fertility increase during the transition period from conventional to green agrarian technologies development. Also, it could be used in zones of technogenic pollution, including in demilitarized zones, areas affected by industrial activity, or exclusion zones of the abandoned mines and factories, etc. [39].

Prospectives of Application in Functionalization of Foods: *P. fluorescens* enzymatic profile suggests potential utility in the functionalization of fruits and vegetables, through the modulation of their metabolism during the cultivation by the shifts in biosynthesis of pigments, alkaloid, etc. bioactive compound with target effects [40]. Potentially, it might offer the breeding of some hypoallergenic varieties of fruits and vegetables. Thus, *P. fluorescens'* presence in environment during cultivation offers several advantages. It not only suppresses the growth of phytopathogens but also induces native defense mechanisms of plants. Specifically, *P. fluorescens* induces systemic resistance in plants, boosting their immunity, mediated by some secondary metabolites: antioxidants (antioxidant status increase), vitamins, anthocyanins, lycopene, etc. For example, the increased levels of lycopene, vitamin C and other antioxidants are shown for *Solanum lycopersicum* (tomatoes), cultivated with *P. fluorescens*. Its presence in rhizosphere of *Rubus fruticosus* (blackberry) activates the genes responsible for the antioxidant status increase by the flavonoids and anthocyanins biosynthesis upregulation [41]. Additionally, *P. fluorescens* produces Fe-chelating compounds (siderophores), pyoverdine and pyochelin, which enhance iron uptake by plants. This increases Fe content in the edible parts, effectively addressing Fe deficiency, as an important part of plant protection. It also may induce biosynthesis of phytohormones (auxins, cytokinins, gibberellins), which stimulates overall plant growth and fruiting, as well as increase the content of valuable nutrients in fruits. Thus, *P. fluorescence* might offer the targeted functionalization of crops by increasing the content of specific bioactive compounds in them. Their consumption has the potential to be beneficial in cases of anemia, vitamin deficiencies, and other nutrient insufficiencies [42].

In addition, *P. fluorescens* synthesizes 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, and phenazines, which inhibit pathogenic microflora not only in the soil, but also on various parts of plants during its development. Thus, food functionalization by the influence of *P. fluorescens* is also possible through its impact on the post-germination microbiota. The presence of *P. fluorescens* influences the surface microflora of fruits, what inhibits the spoilage, promotes natural fermentation (if the product undergoes fermentation during the manufacturing process). Also, it promotes the formation of probiotic coating that may be beneficial when consumed [4-,44].

Thus, *P. fluorescens* application might offer the functional microbiome formation that enhances fruit resistance to spoilage and extends the shelf life and transportability of agricultural products (e.g.: combating green mold in citrus fruits, caused by *Penicillium digitatum*) [45].

Moreover, *P. fluorescens* reduces the residual concentrations of certain pesticides and fertilizers remaining in the soil from previous applications because of biodegradation capabilities [46-49]. Also, it's able to turn the residual concentrations of Zn, Fe, Ag, and Mn in soil into green nano-particles with beneficial properties, what additionally fertilizes the cultivating plants [50-51].

CONCLUSION.

P. fluorescens application as an eco-friendly antiphytopathogenic biocontrol agent holds a significant potential for food quality improvement and its targeted functionalization of fruits and vegetables. The strains from RA collection have a significant potential as novel pest control green agent, useful also for soil remediation and re-fertilization. Thus, *P. fluorescens* is recommended for further detailed research as potential green agent of complex beneficial influence on crops.

Abbreviations: Amc, Amoxiclav; Ampicillin/Amp; AMR, antimicrobial resistance; Amx, Amoxicillin; Azm, Azithromycin; Cam, Chloramphenicol; Cfx, Cefixime; Cip, Ciprofloxacin; Cro, Ceftriaxone; DAPG, 2,4-diacetylphloroglucinol; FTIR-ATR, Fourier Transform Infrared Spectroscopy by Attenuated Total Reflectance; Gnc, Gentamicin; Kan, Kanamycin; Lfx, Levofloxacin; MDC, Microbial Depository; NAS RA, National Academy of Sciences, Republic of Armenia; Pcn, Penicillin; PPO, polyphenol oxidases; SPC, Scientific and Production Center; Stp/Streptomycin; Tcn, Tetracycline; L-Tyr, L-Tyrosine; UV/Vis – Ultraviolet and Visual.

Author's Contributions: All authors contributed to this study.

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REFERENCE

1. Thakur M, Ferrante C, Sanches Silva A. Editorial: Sustainable development in the agro-food and nutrition sector. Front Nutr. 2024, 11:1419937. DOI: https://doi.org/10.3389/fnut.2024.1419937.
2. Thakur M, Prieto MA, Huang H. Editorial: Agri-food innovations in the quest for food and environmental security. Front Nutr. 2023, 10:1304246. DOI: https://doi.org/10.3389/fnut.2023.1304246.
3. Mirzabaev A, Olsson L, Kerr, RB, Pradhan, P., Ferre, MGR., Lotze-Campen H. Climate Change and Food Systems. In: von Braun, J., Afsana, K., Fresco, L.O., Hassan, M.H.A. (eds) Science and Innovations for Food Systems Transformation. Springer, Cham. 2023. 5-27. DOI: https://doi.org/10.1007/978-3-031-15703-5_27.
4. Hounsou EK, Sonibare MA, Elufioye TO Climate change and the future of medicinal plants research. BCHD. 2024, 7(3): 152-169. DOI: https://doi.org/10.31989/bchd.v7i3.1310.
5. Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S, Mohapatra A, Pandey V, Rana N, Cunill JM. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Front Microbiol. 2022, 13:962619. DOI: https://doi.org/10.3389/fmicb.2022.962619.
6. Degani O. Plant Fungal Diseases and Crop Protection. J Fungi (Basel). 2025, 11(4):274. DOI: https://doi.org/10.3390/jof11040274.
7. Melkumyan M, Babayan B, Grigoryan A, Yesayan A. Crops Biological Protection: Phytopathogens Growth Inhibition by The Entomopathogens. BCHD 2024, 7(8): 361- 374. DOI: https://doi.org/10.31989/bchd.v7i8.1427.
8. Yesayan A, Yesayan T, Babayan B, Esoyan S, Hayrapetyan A, Sevoyan G, Chakhmakhchyan A, Nanagulyan S, Melkumyan M. Carnivorous Fungi Application for Pesticide-free Vegetable Cultivation. FFS 2024, 4(9): 325-336. DOI: https://doi.org/10.31989/ffs.v4i9.1432.
9. Chong CE, Pham TM, Carey ME, Wee BA, Taouk ML, Grad YH, Baker KS. Conf. report of the 2024 AMR Meeting. NPJ Antimicrob Resist. 2024, 2(1):43. DOI: https://doi.org/10.1038/s44259-024-00058-z.
10. Riseh RS, Fathi F, Vazvani MG, Tarkka MT. Plant Colonization by Biocontrol Bacteria and Improved Plant Health: A Review. Front Biosci, 2025, 30(1):23223. DOI: https://doi.org/10.31083/fbl23223.
11. Malik D, Kumar S, Sindhu SS. Unlocking the potential of ecofriendly guardians for biological control of plant diseases, crop protection and production in sustainable agriculture. 3 Biotech. 2025;15(4):82. DOI: https://doi.org/10.1007/s13205-025-04243-3.
12. le Coutre J. Editorial: Food, nutrition, and diets at net zero. 10 years of Frontiers in Nutrition. Front Nutr. 2025, 12:1600417. DOI: https://doi.org/10.3389/fnut.2025.1600417.
13. Howells G, Sezmis AL, Blake C, McDonald MJ. Co-Existence Slows Diversification in Experimental Populations of *E. coli* and *P.fluorescens*. Env. Microb. 2025, 27(2): e70061. DOI: https://doi.org/10.1111/1462-2920.70061.

14. Silverio MP, Schultz J, Parise MTD, et al. Genomic and phenotypic insight into AMR of *P. fluorescens* from King George Island, Antarctica. Front Microbiol. 2025, 16:1535420. DOI: https://doi.org/10.3389/fmicb.2025.1535420.
15. Chinmay J, Viren Z, Amit P, Harpal Z., Vibhakshi Z, Prakash Z, Trivedi N S Impact of *P. fluorescens* on Field Pathogen: A Commercial Biofungicide Study. Acta Scientific Microbiology. 2024, 7(7), 58-62. DOI: https://doi.org/10.31080/ASMI.2024.07.1393.
16. Garcia-Seco D, Zhang Y, Gutierrez-Mañero FJ, Martin C, Ramos-Solano B. Application of Pseudomonas fluorescens to Blackberry under Field Conditions Improves Fruit Quality by Modifying Flavonoid Metabolism. PLOS One, 2015, 11, 1-13. DOI: https://doi.org/10.1371/journal.pone.0142639.
17. Mikaelyan AR, Babayan BG, Grigoryan AL, Grigoryan AM, Asatryan NL, Melkumyan MA. Tartaric acid new derivatives as prospective and safe alternative to antimicrobials for food products packing. FFHD 2024, 14(1):33-50. DOI: https://www.doi.org/10.31989/ffhd.v14i1.1195.
18. Martirosyan HS, Mikaelyan AR, Asatryan NL, Babayan BG Assessment of 'Complex–Co' Preparation Efficiency for Some Cereal Crops Growth Stimulation. Agronomy Research 2023, 21(3), 1213–1220. DOI: https://doi.org/10.15159/AR.23.105.
19. Nguyen HB, Phan TL, Ung TT, Nguyen TK. Disc Diffusion Reader: an AI-powered potential solution to combat antibiotic resistance in developing countries. J Infect Dev Ctries. 2025, 19(5):699-711. DOI: https://doi.org/10.3855/jidc.21108.
20. Florez-Cardona V, Khani J, McNutt E, Manta B, Berkmen M. Plasmid Library Construction from Genomic DNA. Curr Protoc. 2025, 5(1): e70088. DOI: https://doi.org/10.1002/cpz1.70088.
21. Sánchez-Flores JE, Sandoval-Cabrera A, Alarcón-Valdés P, Santillán-Benítez JG An affordable and simple method for DNA extraction from agarose suitable for downstream applications. Sci Rep. 2025, 15,10414. DOI: https://doi.org/10.1038/s41598-025-87572-w.
22. Meitil IKS, de O G Silva C, Pedersen AG, Agger JW. Classification of PPO shows ancient gene duplication leading to two distinct enzyme types. iScience. 2025, 28(2):111771. DOI: https://doi.org/10.1016/j.isci.2025.111771.
23. McGivern BB, Cronin DR, Ellenbogen JB, Borton MA, Knutson EL, Freire-Zapata V, Bouranis JA, Bernhardt L, Tfaily MM, Sullivan MB, Tyson GW, Rich VI, Hagerman AE, Wrighton KC Microbial polyphenol metabolism is part of the thawing permafrost carbon cycle. Nat Micr. 2024, 9:1454-1466. DOI: https://doi.org/10.1038/s41564-024-01691-0.
24. Huang J, Chien MF, Bacosa HP, Inoue C. Production of lipase and extracellular polymeric substances by the lipid-degrading *B. arboris* strain JYK2 in response to different substrates. World J Micr. Bio 2025, 41(6):198. DOI: https://doi.org/10.1007/s11274-025-04432-5.
25. Aryal B, Lehtimaki M, Rao VA. Stress-mediated polysorbate 20 degradation and its potential impact on therapeutic proteins. Pharm Res. 2024, 41(6):1217-1232. DOI: https://doi.org/10.1007/s11095-024-03700-7.
26. Melkumyan M, Bababyan B. The Study of Proteolytic Activity of Some Strains of *P. carotovorum* Representatives Phytopathogenic Bacteria. Bulletin of High Technology, 2024, 4(32), 3-10. DOI: https://doi.org/10.56243/18294898-2024.4-3.
27. Melkumyan M, Babayan B. The Study of Proteolytic Activity of Phytopathogenic *Rhodococcus, Pseudomonas* and *Xanthomonas* Some Strains. Eur. Sci. Review. 2023, 1-2(1): 3-7 DOI: https://doi.org/10.29013/ESR-23-1.2-3-7.
28. Pickett MJ, Greenwood JR, Harvey SM. Tests for detecting degradation of gelatin: comparison of five methods. J Clin Microbiol. 1991, 29(10):2322-5. DOI: https://doi.org/10.1128/jcm.29.10.2322-2325.1991.
29. Selegato DM, Castro-Gamboa I. Enhancing chemical and biodiversity by co-cultivation. Front.Micr.2023,14: 1117559. DOI: https://doi.org/10.3389/fmicb.2023.1117559.
30. Aida H, Ying BW. Population Dynamics of *E. coli* Growing under Chemically Defined Media. Sci Data. 2025, 12(1):984. DOI: https://doi.org/10.1038/s41597-025-05356-3.
31. Dróżdż A, Kubera D, Olender A, Dabrowski W, Szukala M, Wosko S, Chwiej J, Rugiel M, Kawoń K, Gagoś M. ATR-FTIR markers indicating drug resistance in selected *Candida* strains. Sci Rep.2025,15(1):18130. DOI: https://doi.org/10.1038/s41598-025-01428-x.
32. Abady KK, Karpourazar N, Krishnamoorthi A, Li R, Rentzepis PM. Spectroscopic analysis of bacterial photoreactivation. Photochem Photobiol. 2025, 101(2):494-504. DOI: https://doi.org/10.1111/php.14019.
33. Feldl M, Olayo-Alarcon R, Amstalden MK, Zannoni A, Peschel S, Sharma CM, Brochado AR, Müller CL. Statistical end-to-end analysis of large-scale microbial growth data with DGrowthR. bioRxiv 2025, 03(25), 645164: 1-18. DOI: https://doi.org/10.1101/2025.03.25.645164.
34. Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics. 2017, 18(1):529. DOI: https://doi.org/10.1186/s12859-017-1934-z.

35. Gutiérrez EJ, Abraham MDR, Baltazar JC, Vázquez G, Delgadillo E, Tirado D. *P. fluorescens*: A Bioaugmentation Strategy for Oil-Contaminated and Nutrient-Poor Soil. Int J Env. Res Public Health. 2020, 17(19):6959. DOI: https://doi.org/10.3390/ijerph17196959.
36. Selvan SA, Ghalsasi VV, Bandyopadhyay A, Pal T. Commercial utilization of bacteriocins: tackling challenges and exploring their potential as alternatives to antibiotics. Future Microbiol. 2025, 1-13. DOI: https://doi.org/10.1080/17460913.2025.2520693.
37. Liu P, Wen S, Zhu S, Hu X, Wang Y. Microbial Degradation of Soil Organic Pollutants: Mechanisms, Challenges, and Advances in Forest Ecosystem Management. Processes. 2025, 13(3):916. DOI: https://doi.org/10.3390/pr13030916.
38. Tilley A, McHenry MP, McHenry JA, Solah V, Bayliss K. Enzymatic browning: The role of substrates in PPO mediated browning. Curr Res Food Sci. 2023, 7:100623. DOI: https://doi.org/10.1016/j.crfs.2023.100623.
39. Sabaghi M, Maleki SJ. Mitigating Food Protein Allergenicity with Biopolymers, Bioactive Compounds, and Enzymes. Allergies. 2024, 4(4):234-253. DOI: https://doi.org/10.3390/allergies4040016.
40. Naga MYA, Khan MA, Abu-Hussien SH, Mahdy SM, Al-Farga A, Hegazy AA. Optimizing lipase production by Bacillus subtilis on cheese whey and evaluating its antimicrobial, antibiofilm, anti-virulence and biosafety properties. Sci Rep. 2025 Apr 1;15(1):11087. DOI: https://doi.org/10.1038/s41598-025-92181-8.
41. Tang Q, Li Z, Chen N, Luo X, Zhao Q. Natural pigments derived from plants and microorganisms: classification, biosynthesis, and applications. Plant Biotechnol J. 2025, 23(2):592-614. DOI: https://doi.org/10.1111/pbi.14522.
42. Wang K, Yang Z, Luo S, Quan W. Endophytic *P. fluorescens* promotes changes in the phenotype and secondary metabolite profile of Houttuynia cordata Thunb. Sci Rep. 2024, 14(1):1710. DOI: https://doi.org/10.1038/s41598-024-52070-y.
43. Mishra, S., Jha, C.K., Goswami, D., Saraf, M. *P. fluorescens*: An Influencer for Food Crop Biofortification by Siderophore-Mediated Iron Acquisition. In: Pandey, P., Dheeman, S., Maheshwari, D.K. (eds) Microorganisms Resilience to Climate Change. Micr for Sust. 2025, 56, 225-245. DOI: https://doi.org/10.1007/978-981-96-3748-5_10.
44. Manshin D, Kuntsova M, ShilenkoA, Andreeva A. Probiotic yeast *S. cerevisiae var. boulardii*: properties and peculiarities of use in functional foods development. FFHD. 2025; 15(5): 295-315. DOI: https://doi.org/10.31989/ffhd.v15i5.1482.
45. Wang Zh, Mengyao J, Kewei C, Wang K, Du, M, Zalán Z, Hegyi F, Kan J, Biocontrol of Penicillium digitatum on Postharvest Citrus Fruits by Pseudomonas fluorescens, Journal of Food Quality, 2018, 2910481,1-10, DOI: https://doi.org/10.1155/2018/2910481.
46. Jalal A, Oliveira CEDS, Fernandes GC, da Silva EC, da Costa KN, de Souza JS, Leite GDS, Biagini ALC, Galindo FS, Teixeira Filho MCM. Integrated use of plant growth-promoting bacteria and nano-Zn foliar spray is a sustainable approach for wheat biofortification, yield, and Zn use efficiency. Front Plant Sci. 2023, 14:1146808. DOI: https://doi.org/10.3389/fpls.2023.1146808.
47. Essawy E, Abdelfattah MS, El-Matbouli M, Saleh M. Synergistic Effect of Biosynthesized Silver Nanoparticles and Natural Phenolic Compounds against Drug-Resistant Fish Pathogens and Their Cytotoxicity: An In Vitro Study. Mar Drugs. 2021, 19(1):22. DOI: https://doi.org/10.3390/md19010022.
48. Ashrafi-Saiedlou S, Rasouli-Sadaghiani M, Fattahi M, Ghosta Y. Biosynthesis and characterization of iron oxide nanoparticles fabricated using cell-free supernatant of *P. fluorescens* for antibacterial, antifungal, antioxidant, and photocatalytic applications. Sci Rep. 2025, 15(1):1018. DOI: https://doi.org/10.1038/s41598-024-84974-0.
49. Selim S, Abdelghany TM, Almuhayawi MS, Elamir MYM, Alharbi AA, Al Jaouni SK Biosynthesis and activity of Zn-MnO nanocomposite in vitro with molecular docking studies against multidrug resistance bacteria and inflammatory activators. 2025, Sci Rep 15, 2032. DOI: https://doi.org/10.1038/s41598-024-85005-8.
50. Ismayawati AD, Yokoyama D, Goto R, Oya Y, Watanabe I, Horai S, Sakakibara H. Mineral composition and antioxidant activity of partially digested coffee "KopiLuwak". FFHD, 2024; 14(11): 801-813. DOI: https://doi.org/10.31989/ffhd.v14i11.1469.
51. Panagiotou T, Fisher RJ. Producing micron- and nano-size formulations for functional foods applications. FFHD, 2013, 3(7):274-289. DOI: https://doi.org/10.31989/ffhd.v3i7.48.