

Isolation of Bacteria Producing Antifungal Substances from Aquatic Environments

Nassira Tabli* and El-hafid Nabti

Renewable Energy Management Laboratories, Faculty of Natural and Life Sciences, University of Bejaia, Algeria

*Corresponding Author: Nassira Tabli, Renewable Energy Management Laboratories, Faculty of Natural and Life Sciences, University of Bejaia, Algeria.

Received: January 14, 2020; Published: February 13, 2020

Abstract

The interest aroused in bacteria from irrigation wells results from their involvement in maintaining soil health and the good development of crops. 48 bacterial isolates are purified from water from a well located in northern Algeria. 4 strains are selected based on their inhibitory effect on two phytopathogenic fungi, as well as their ability to express a few traits promoting plant growth, namely; indol acetic acid, lytic enzymes, phosphate solubilization, production of iron fixing siderophores, etc. Following the sequencing of rR16S, the strains; B, D and N are affiliated with *Pseudomonas* protegens, while C is a *Serratia* carnivoran. The growth inhibition percentages for *Botrytis cinerea* and *Aspergillus niger* range from 60 to 90%. The strains produce volatile compounds with PGI% ranging from 13 to 50%. An *in vivo* greenhouse test is carried out to determine the stimulating effect of the 4 isolates on the growth of tomato and pea plants, and the protection of the latter in the presence of *Pythium aphanidermatum* spores, the N strain significantly improved the germination of peas (+ 25%) and their fresh weight (+ 43%), as well as the fresh weight of the tomato stems (+ 10%). These results support the use of PGPB as growth promoters and protectors for plant health.

Keywords: *Pseudomonas*; Aquatic Bacteria; Biocontrol Agents; PGPB

Introduction

The global population explosion, and the continuous degradation of the environment have worrying consequences on food production, which becomes insufficient to feed this growing population. This decrease in production is due to certain abiotic and biotic factors [1]. The biotic factors represented by plant diseases caused by bacteria, fungi, viruses, and nematodes are considered a major problem reducing the final yield of crops and post-harvest losses (Borges., *et al.* 2014). It has therefore become essential to increase agricultural production and minimize losses by looking for alternative methods [2]. With intensive agricultural production, producers have become increasingly dependent on chemical synthesis products to protect crops [3]. Unfortunately the increasing (increased) use of chemical inputs has serious consequences, namely; resistance to pathogens, their accumulation in the environment, and the appearance of harmful effects on other non-target organisms [4]. The use of PGPB as growth promoters and crop protectants is increasingly documented [5-7]. These bacteria act through direct and indirect mechanisms. The genera *Bacillus* and *Pseudomonas* are the most studied for their various PGP and biocontrol traits [8].

Material and Methods

Water sampling

The water samples are taken from an agricultural well in northern Algeria; Djebira village (36° 41'59.2 "N 5° 04'28.8" E). The bacteria are isolated and purified on PCA agar and stored for later testing.

The antifungal test

48 bacterial isolates are tested for *A. niger* and *B. cinerea* according to the protocol of Sagahón., *et al.* [9]. The dishes are incubated at 25 ± 2°C for 5 days, the percentages of inhibition of mycelial growth (PGI%) are calculated. The four best isolates selected from the previous test are phylogenetically identified by sequencing their rR16s [10,11].

Research of some PGP and biocontrol traits

- **Inhibition of spore germination:** The ability of bacterial isolates to inhibit the growth of the two fungi (*A. niger* and *B. cinerea*) is tested through their ability to inhibit the germination of spores. The protocol followed is that of Sadfi-Zouaoui., *et al.* [12], and the results are expressed by the calculation of the percentage of inhibition of spore germination (SGI%).
- **Synthesis of volatile antifungal substances:** The production of volatile compounds involved in the antagonistic activities is tested according to the protocol described by Dennis and Webster [13], the radial fungal growth is recorded 7 days later, and the PGI% values are obtained by comparing to the control.

Metabolic and functional characterization

- **Lytic enzymes:** The search for hydrolytic enzymes is carried out using the agar diffusion method, by depositing a 5mm disc of the bacterial culture on agar medium containing the substrate for the desired enzyme. The activities highlighted are: cellulase [14], esterase [15], lipase [15], chitinase [16], protease [15], amylase [17] and urease [18].
- **Solubilization of phosphorus:** The test is carried out on Pikovskaya agar medium, as described by Peix., *et al.* [19]. A positive result is reflected in the appearance of a transparent halo around the colonies.
- **Production of siderophores:** The search for iron fixing siderophores is carried out on agar medium Chrom Azurol-S, according to the protocol of Schwyn and Neilands [20].
- **AIA synthesis:** The production of this phytohormone is determined according to the protocol of Bric., *et al.* [21]. The assay is spectrophotometric (530 nm), and the results are obtained by extrapolation on a calibration curve.

In vivo tests

- **Promotional effect of tomato growth:** The 4 bacterial isolates served as an inoculum, and are tested on tomato plants in pots in a greenhouse. The strains of *Pseudomonas protegens* CHA0, and *Pseudomonas* sp DSMZ13134 are used in the experiment as reference strains, the detailed protocol is that of Tabli., *et al.* [22].
- **Control of damping-off of seedlings on pea seeds:** The protocol is that followed by Tabli., *et al.* [22]. As for the tomato test, the isolates; B, C, D and N are the test strains in soil inoculated with spores of *Pythium aphanidermatum*, and the strains of *Pseudomonas protegens* CHA0, and *Pseudomonas* sp DSMZ13134 are the reference strains. 60 days after sowing the percentage of emergence of the plants and their total fresh weight are measured.

Results and Discussion

Inhibition of mycelial growth

Of the 48 isolates, the strains; B, C, D and N appeared to be the most effective of the two phytopathogens tested. B and N showed the greatest activity towards *A. niger* (90%), and around 68% for isolates C and D. isolate a D shows 82.5% inhibition against *B. cinerea*. The isolates N, C and B showed PGI% ranging from 63% (B), to 80% (N).

Inhibition of spore germination

The presence of the 4 strains reduced *A. niger* growth by 20%, while for *B. cinerea* the inhibition is total (100%).

Phylogenetic identification

Phylogenetic analysis showed that the rRs16 gene sequences of isolates B, D and N are the closest to *Pseudomonas protegens* CHA0 (99.93%), while isolate C exhibits the highest similarity (99.86%) with *Serratia* carnivorans.

PGP and biocontrol features

The four strains produce the enzymes; lipase, chitinase, esterase and urease, but not Amylase, strain C is the only phosphorus solubilizer. All of the strains synthesize siderophores, and produce ammonia. Strains C and N produce more HCN compared to B and D. The values of AIA vary between 2.40 µg/ml ± 0.65 and 5.09 µg/ml ± 0.65 as the minimum and maximum value produced by strains C and D respectively.

Synthesis of volatile substances

PGI% values for *A. niger* following the production of volatile compounds by strains B, C, D and N vary between 8 and 22%. Strain C showed the best inhibitory effect (50.8%) against *B. cinerea* compared to the other strains.

Growth promoting effect on tomato plants

The strain C significantly increased the fresh weight (+ 15%) compared to the non-inoculated controls, the growth was better than the other strains tested including those of references CHA0 and DSMZ13134.

Pythium aphanidermatum control on peas

Infection with *Pythium aphanidermatum* reduced the emergence of peas from 83.3% in the uninoculated control to 7.5% in the soil inoculated with the pathogen. All the isolates except for isolate D reduced the damping-off of the seedlings but remains significant just for the strain N, the emergence is 26.3% (7 times compared to the control) than the reference strains CHA0 and DSMZ13134 which stimulated the emergence of 43 and 2 times respectively. Strains B and C did not show a significant difference in emergence compared to the control. In the soil inoculated with the fungus the weight is reduced from 05g/plant (control) to 0.23g. The isolate N and CHA0 alone stimulated the fresh weight with 43% and 48%, no increase in weight was recorded for the rest of the strains (DSMZ13134, D, C, B).

Discussion

Following the serious problems caused by chemical pesticides, the application of antagonistic microorganisms or their metabolites is another alternative. The bacteria isolated in this present work are known for their inhibitory effect on phytopathogens by several mechanisms [23-25]. According to Rai, *et al.* [24], a strain of *Pseudomonas protegens* RhiNA inhibited the mycelial growth of *A. flavus*, *B. cinerea*, *A. niger* and *Mucor* sp. This activity is attributed to the production of various metabolites. *Pseudomonas* strains produce antibiotics, phytohormones, lytic enzymes and siderophores [26,27]. The isolates tested in this work produce these metabolites, which may explain their effectiveness in stimulating the growth of tomatoes and peas. Extracellular enzymes are also known for their role in biocontrol, certain bacterial enzymes destroy spores, prevent their germination, and inhibit mycelial growth [28]. Suresh, *et al.* [29] and Raaijmakers, *et al.* [30] reported the PGP role of *Pseudomonas* through the synthesis of HCN, Siderophores, proteases, antimicrobial agents and solubilization of phosphorus.

Serratia stimulated pea germination by 83% in the presence of *P. aphanidermatum* and tomato growth by 15%, such PGP traits and biocontrol can be explained by their capacity to produce AIA phytohormone, siderophores, solubilization of phosphorus and degradation of chitin [30]. Volatile compounds (NH₃ and HCN) can also be the cause of inhibition of mycelial growth [31]. Ponmurugan and Gopi [32] reported the role of *Pseudomonas*-produced AIA in stimulating the root development of small peas. According to Kraus and Loper [33], a

strain of *Pseudomonas* stimulated cucumber germination in the presence of *Pythium* through competition for nutrients and the secretion of antifungal metabolites such as siderophores and chitinases. The inhibitory effect of the N strain on *pythium* can be attributed to the production of siderophores, proteases, chitinases and AIA [34,35].

Conclusion

Water from irrigation wells has become a source of bacteria of agricultural interest. We managed to isolate 3 strains of *Pseudomonas* and a strain of *Serratia* which we selected on the basis of preliminary tests of growth promotion and biocontrol through inhibition of the germination of spores and that of mycelial growth. These effects also appeared once these bacterial isolates are applied *in vivo* on tomato plants and pea seeds, the parameters; emergence and fresh weight are measured in the presence and absence of *P. aphanidermatum*. These results make it possible to consider irrigation well water as a source of bacteria with a growth stimulating role and an inhibitor of phytopathogens.

Bibliography

1. El Khoury, *et al.* "Integrated plant disease management in developing countries". *Journal of Plant Pathology* 92.4 (2010): S4.35-S4.42.
2. Glick BRBR. "Plant Growth-Promoting Bacteria: Mechanisms and Applications". *Hindawi Publishing Corporation Scientifica* (2012).
3. Thakur DS., *et al.* "Role of Fluorescent *Pseudomonas* in reduction of the use of chemical pesticides and fungicides in normal and re-plant sites of apple and pear". *Asian Journal of Biological Sciences* 8.2 (2013): 259-266.
4. Belhadi A., *et al.* "Pratiques Phytosanitaires des serrotes maraichers de trois localités de l'est des Ziban et leur impact potentiel sur la santé humaine et l'environnement". *Review of Agricultural* 1 (2016): 09-16.
5. Jeon JS., *et al.* "Plant Growth Promotion in Soil by Some Inoculated Microorganisms". *Journal of Microbiology* 41.4 (2003): 271-276.
6. Tabli N., *et al.* "Impact of diazotrophic bacteria on germination and growth of tomato, with bio-control effect, isolated from Algerian Soil". *Journal of Environmental Health Science and Engineering* 1 (2014): 1-7.
7. Noumavo PA., *et al.* "Plant Growth Promoting Rhizobacteria: Beneficial effects for healthy and sustainable agriculture". *African Journal of Biotechnology* 15.27 (2016): 1452-1463.
8. Sheeba J., *et al.* "Isolation, Screening and Characterization of Plant Growth Promoting Bacteria and their Antifungal Effect on *Rhizoctonia Solani* (J.G. Kühn 1858)". *Advances in Plants and Agriculture Research* 7.5 (2017): 00269.
9. Sagahón IP., *et al.* "Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*". *International Journal of Molecular Sciences* 12 (2011): 5522-5537.
10. Edwards U., *et al.* "Isolation and direct complete nucleotide determination of entire genes: characterization of a gene coding for 16S ribosomal RNA". *Nucleic Acids Research* 17 (1989): 7843-7853.
11. Stackebrandt E. and Liesack W. "Nucleic acids and classification". In M. Goodfellow and A.G.O'Donnell (eds.), *Handbook of new bacterial systematics* Academic Press, London, England (1993): 152-189.
12. Sadfi-Zouaoui N., *et al.* "Biological control of *Botrytis cinerea* on stem wounds with moderately halophilic bacteria". *World Journal of Microbiology and Biotechnology* 24 (2008): 2871-2877.
13. Dennis C and Webster J. "Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics". *Transactions of the British Mycological Society* 57(1971): 363-369.

14. Carder JH. "Detection and condition of cellulase by Congo Red staining of substrates in a cup plate diffusion assay". *Analytical Biochemistry* 153 (1986): 75-79.
15. Carrim AJI, et al. "Enzymatic activity of endophytic bacterial isolates of Jacaranda decurrens Cham. (Carobinha-do-campo)". *Brazilian Archives of Biology and Technology* 49 (2006): 353-359.
16. Kopečný J., et al. "The isolation and characterization of a rumen chitinolytic bacterium". *Letters in Applied Microbiology* 23 (1996): 195-198.
17. Vinoth RS., et al. "Study of starch degrading bacteria from kitchen waste soil in the production of amylase by using paddy straw". *Recent Research in Science and Technology* 1 (2009): 008-013.
18. Christensen WB. "Urea decomposition as a means of differentiating proteus and paracolon cultures from each other and from Salmonella and Shigella types". *Journal of Bacteriology* 52 (1946): 461-466.
19. Peix A., et al. "Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions". *Soil Biology and Biochemistry* 33 (2001): 103-110.
20. Schwyn B and Neilands JB. "Universal chemical assay for the detection and determination of siderophores". *Analytical Biochemistry* 160 (1987): 47-56.
21. Bric JM., et al. "Rapid in situ assay for indole acetic acid production by bacteria immobilization on a nitrocellulose membrane". *Applied and Environmental Microbiology* 57 (1991): 535-538.
22. Tabli N., et al. "Plant growth promoting and inducible antifungal activities of irrigation well water-bacteria". *Biological Control* 117 (2018): 78-86.
23. Bensidhoum L., et al. "Heavy metal tolerant *Pseudomonas protegens* isolates from agricultural well water in north eastern Algeria with plant growth promoting, insecticidal and antifungal activities". *European Journal of Soil Biology* 75 (2016): 38-46.
24. Rai A., et al. "A *Pseudomonas Protegens* with high antifungal activity protects apple fruits against *Botrytis cinerea* Gray Mold". *International Journal of Scientific Research in Science and Technology* 2 (2016): 227-237.
25. Kamil Z., et al. "Isolation and identification of rhizosphere soil chitinolytic bacteria and their potential in antifungal biocontrol". *Global Science Research Journals* 2 (2007): 57-66.
26. Tri Wahyudi A., et al. "Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting Rhizobacteria". *Journal of Microbiology and Antimicrobials* 3 (2011): 34-40.
27. Bouizgarne B. "Bacteria for Plant Growth Promotion and Disease Management". In: Maheshwari, D.K. (Eds.), *Bacteria in Agrobiolgy: Disease Management* (2013): 15-47.
28. Suresh A., et al. "Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants". *African Journal of Microbiology Research* 4.14 (2010): 1491-1494.
29. Raaijmakers JM., et al. "Antibiotic production by bacterial biocontrol agents". *Antonie van Leeuwenhoek* 81 (2002): 537-547.
30. Brurberg MB., et al. "Comparative studies of chitinases A and B from *Serratia marcescens*". *Microbiology* 142 (1996): 1581-1589.

31. Trivedi P, *et al.* "In vitro evaluation of antagonistic properties of *Pseudomonas corrugate*". *Microbiological Research* 163 (2008): 329-336.
32. Ponmurugan P and Gopi C. "In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria". *African Journal of Biotechnology* 54 (2006): 348-350.
33. Kraus J and Loper JE. "Lack of evidence for a role of antifungal metabolite production by *pseudomonas fluorescens* Pf-5 in biological control of pythium damping off of cucumber". *Molecular Plant Pathology* 82 (1992): 264-271.
34. Khabbaz SE and Abbasi PA. "Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber". *Canadian Journal of Microbiology* 60 (2014): 25-33.
35. Nzungize JR, *et al.* "Pythium root rot of common bean: biology and control methods. A review". *Biotechnology, Agronomy, Society and Environment* 3 (2012): 405-413.

Volume 15 Issue 3 March 2020

©All rights reserved by Nassira Tabli and El-hafid Nabti.