Application of aquaponic microorganisms alone or in consortium as original biocontrol method of lettuce root rots in soilless culture

G. Stouvenakers, S. Massart and M.H. Jijakli

Laboratory of Integrated and Urban Phytopathology, University of Liège, Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium.

Abstract

Root pathogens in hydroponic culture are often difficult to control without the use of synthetic pesticides. Moreover, most of the available biocontrol agents were isolated from soils. They were not developed for soilless application and often resulted in poor efficacy. It is therefore important to find novel sources of beneficial microorganisms that could grow and protect plant root in such aquatic environment. In this context, aquaponic systems that combines hydroponic plant culture and fish farming were described as a promising source of biocontrol agents. From a previous study, three aquaponic microorganisms were isolated and selected to evaluate their capacity to control *P. aphanidermatum* root rot disease on lettuce. *Sphingobium xenophagum* SHb30, *Mycolicibacterium fortuitum* C13 and *Aspergillus flavus* G2 were the three strains used alone or in consortium to protect soilless lettuce. Treatments were compared with a biocontrol agent registered against *Pythium* diseases, a propamocarb fungicide and the direct use of aquaponic water. G2 treatment alone protected lettuce as well as the fungicide and the foliar fresh mass of lettuce was similar to healthy lettuce. C13 had no effect on the disease, while SHb30 limited foliar yield loss. Consortium containing G2 gave similar results than G2 alone but the SH30+C13 combination tended to increase the protective effect in comparison with separated application. This study highlighted that aquaponic water or some of its microorganisms applied alone or in consortium could control *P. aphanidermatum* root rot disease on lettuce in soilless conditions in a similar way than a fungicide and with a better action than a registered biocontrol agent. Further research will aim at developing appropriated formulation to stabilize and improve biocontrol efficacy of these strains.

Keywords: biocontrol, antagonist, consortium, hydroponics, aquaponics, *Pythium aphanidermatum*, lettuce

INTRODUCTION

Commercialized biocontrol agents used to protect plant against root diseases in soilless culture often lack high efficacy. In fact, most biocontrol agents were isolated from soil, then studied and developed for soil uses (Postma et al., 2008; Vallance et al., 2011; Montagne et al., 2017). The consequence is therefore a poor adaptation of these microorganisms to aquatic conditions and parameters met in soilless systems often found under greenhouse structures (Postma et al., 2008; Vallance et al., 2011). Moreover, in these specific conditions, some root pathogens particularly adapted to water can rapidly spread the disease in the system. It is particularly true for *Oomycetes* pathogens that produce flagellated spores, such as *Pythium aphanidermatum* (Edson) Fitzp. This fungal pathogen causes root rot disease on lettuce (Sutton et al., 2006) in hydroponics and aquaponics. For aquaponics, chemical pesticides are inadvisable because of the presence of fish in the same water loop as plants (Stouvenakers et al., 2019). To find novel biocontrol agents adapted to such environment, an isolation campaign of beneficial microorganisms found in hydroponics was led around 1995 and onward, but very few isolates led to commercialization (McPherson et al., 1995; Vallance et al., 2011). Nevertheless, it was recently highlighted that aquaponics could contain original microorganisms able to control *P. aphanidermatum* disease (Stouvenakers et al., 2020). From

the list of potential antagonistic microorganisms set in it, a selective isolation was undertaken by Stouvenakers et al. (2023) and led to 110 isolates. The isolates were then screened to control *P. aphanidermatum* disease on lettuce. In the present study, the 3 most effective isolates detected in the past screening were applied alone or in consortium to control the same plant pathogen on lettuce seedlings.

MATERIALS AND METHODS

Treatments and controls

The three most effective isolates for controlling *P. aphanidermatum* on lettuce identified by Stouvenakers et al. (2023) were selected for this experiment. Two bacteria and one fungus were used alone or in consortium against the pathogen. They were *Sphingobium xenophagum* strain SHb30, *Mycolicibacterium fortuitum* strain C13 and *Aspergillus flavus* strain G2. The two bacteria were produced in liquid rich medium (R medium) that contained in 1 L of distilled water: 10 g peptone, 5 g yeast extract, 5 g malt extract, 5 g bacto-casamino acids, 2 g beef extract, 2 g glycerol and 1 g $MgSO_4$. Bacteria were incubated at 28°C with 100 rpm shaking for 3 days. Bacterial pellets were recovered by culture medium centrifugation at 4000 G for 10 min. Pellets were rinsed with 0.05 M kalium phosphate buffer plus 0.05% Tween 80 (KPBT), centrifuged again and then resuspended in KPBT. Concentration of the suspensions were determined by spectrophotometer set at 600 nm and adjusted to 1×10^9 cfu mL^{-1} in KPBT. G2 fungus was grown in potatoes dextrose agar (PDA, Merck Millipore) and incubated at 23°C for 7 days. Spores were scratched off in KPBT and filtered through cheesecloth. Spore concentration of the filtrate was measured in haemocytometer and fixed at 1×10^8 spores mL^{-1}. Produced bacterial and fungal suspensions were used alone (SHb30, C13 or G2 treatments) or in consortium to treat lettuce in the experiment. Combinations made for consortium treatments were SHb30+C13, SHb30+G2 and SHB30+C13+G2. Mixtures were made with equal proportion of each constituent in described concentration. Controls used were a negative healthy control without the pathogen (C-), a positive control (C+), a biopesticide control (Cpc), an aquaponic control (Cap) and a fungicide control (Cf). C+, C- and Cap were treated with KPBT. Lettuces in Cap were grown in aquaponic water (instead of commercialized hydroponic solution) collected in the PAFFbox aquaponic system of Gembloux Agro Bio-Tech in Belgium (see Stouvenakers et al., 2020 for the system description). For Cf, Proplant® (722 g L^{-1} propamocarbe) fungicide was used at 0.1% in KPBT buffer. Finally, *Pseudomonas chlororaphis* Tx-1 (ATCC 55670) suspension, produced as other bacteria and at the same concentration, was used for Cpc.

Pathogen inoculum preparation

According to Stouvenakers et al. (2023), stock mycelial culture of *Pythium aphanidermatum* (CBS 132490) was first reactivated on PDA for 3 days at 23°C with a day/night photoperiod of 18h/6h. Then, mycelial plugs of the active growing fungus were grown in Erlenmeyer flasks containing 25 mL of clarified V8 CaCO3 broth (800 mL of distilled water, 200 mL of V8 juice, and 3 g of $CaCO_3$). After 9 days at the same conditions, mycelial bulk was recovered and rinsed several times in sterile distilled water. Mycelium bulks were then incubated for 24 h at 28°C with lighting in sterile distilled water to initiate oospores formation and maturation. Mycelium bulks were then mixed with a hand blender (Braun Minipimer Control Plus, 300w) in a sterile solution containing 10 mM of sucrose and 0.05% of Tween 20 in distilled water. Oospores in suspension were then separated from other propagules by sterile cheesecloth filtration. Oospores found in the filtrate were then set at a concentration of 1×10^4 oospores mL^{-1} after haemocytometer observation.

Experimental setup

Biocontrol experiment of *P. aphanidermatum* disease on lettuce seedlings was conducted as described in Stouvenakers et al. (2023). Organic pelleted lettuce seeds (*Lactuca sativa* 'Lucrecia RZ') (Rijk Zwaan, Merksem, Belgium) were sown in 25×25×40 mm rockwool plugs (Grodan B.V., Roermond, The Netherlands). Plugs were put in square plant trays of 14

cm side and trays were then randomly placed in a phytotron set at 16 h/8 h (day/night) photoperiod, a temperature of 22°C/18°C (day/night), and a relative humidity of 65%. Excepted for Cap treatment, where aquaponic water was used all experiment long, tap water was used for the first week of germination and then hydroponic solution was used instead according to manufacturing instruction (Hy-Pro A and B, Hy-Pro Fertilizers, Bladel, The Netherlands). Ten days after sowing, temperatures and humidity were increased to 35/25°C (day/night) and 92%, respectively. Treatments were applied at a rate of 1 mL plug^{-1} on days 0 and 7. For each treatment, 2 plant trays were used containing each 9 rockwool plugs. On day 10 after sowing, plugs were inoculated by 1 mL of the pathogen suspension, excepted for C- where sucrose + tween solution was used instead. Lettuce mortality (LM), root rot symptoms (RRR: root rot rating) and foliar fresh mass (FFM) were recorded on day 31 as described in Stouvenakers et al. (2020). Statistics were achieved for FFM and RRR data on Minitab v.19 software (Minitab Inc., State College, PA, USA). Conditions of application were tested, and 1-way analysis of variance (ANOVA) were performed with treatments as a factor. Tukey multiple comparison post hoc test was used to pairwise compare treatments.

RESULTS

LM means were provided in Table 1. RRR and FFM means were illustrated in Figure 1A and B. The highest LM was observed in C13 treatment and Cpc control (33.3%, respectively), while LM in C+ was 11.1%. Other controls or treatments used alone and in consortium had no LM.

Table 1. Lettuce mortality (LM) of C13, SHb30, G2 treatments applied alone or in consortium to control *P. aphanidermatum* disease on lettuce seedlings. C+, C-, Cf and Cap were the positive, negative, fungicide and biofungicide controls, respectively.

LM: lettuce mortality (%)										
Controls				Treatments						
C-	C+	Cf	Cap	Cpc	C13	SHb30	G2	SHb30+C13	SHb30+G2	SHb30+C13+G2
0.0	11.1	0.0	0.0	33.3	33.3	0.0	0.0	0.0	0.0	0.0

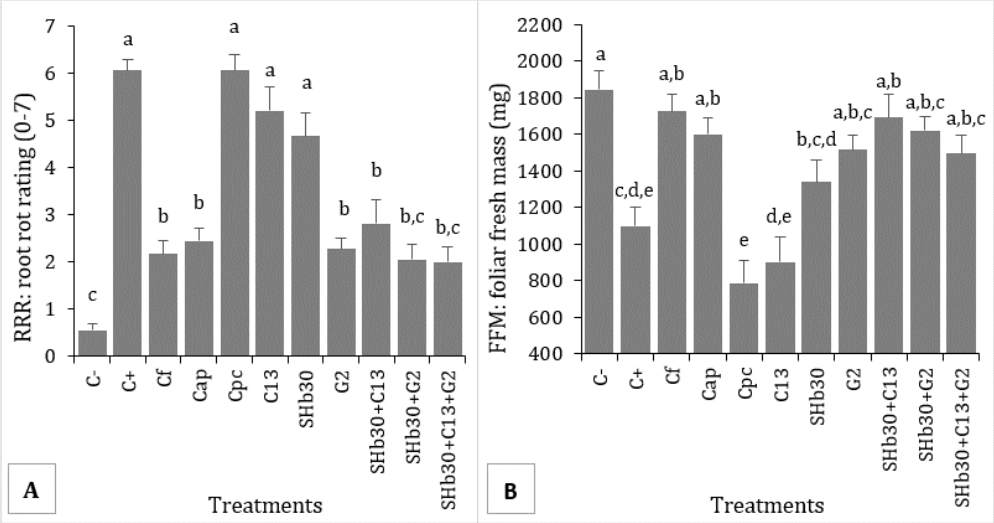

Figure 1. Means bar charts of A: root rot rating (RRR), and B: foliar fresh mass (FFM) of C13, SHb30, G2 treatments applied alone or in consortium to control *P. aphanidermatum* disease on lettuce seedlings. C+, C-, Cf and Cap were the positive, negative, fungicide and biofungicide controls, respectively. Bars indicate the standard error of the mean and different letters indicate significant differences ($p \leq 0.05$) between treatments by Tukey's ANOVA post hoc test.

Although, low LM was observed in C+, disease was present with a RRR=6.06 and a FFM=1100.4 mg for this positive control. In comparison, RRR and FFM of C- were 0.56 and 1844.9 mg, respectively. Cf and Cap controls were effective ($p \leq 0.05$) to reduce RRR (2.17 and 2.14, respectively) and no FFM significant decrease ($p > 0.05$) was observed compared with C-. However, Cpc was not able to control the disease, with a RRR mean of 6.06 and a FFM mean of 788.2 mg. C13 and SHb30 tended to decrease root symptoms (RRR=5.2 and 4.7, respectively) but not significantly ($p > 0.05$). With the combination of the two bacteria (SHb30+C13), RRR dropped down ($p \leq 0.05$) to 2.81 compared with C+. Among treatments applied alone, G2 provided the best ($p \leq 0.05$) root disease protection with a RRR of 2.3, that was not different ($p > 0.05$) from Cf and Cap. All consortiums tested were effective to reduce RRR. In fact, their RRR levels were similar ($p > 0.05$) to RRR of Cf and Cap controls. Moreover, consortiums that contained G2 fungus (SHb30+G2 and SHb30+C13+G2) were not different from RRR of C- and G2 alone. In relation to FFM, C13 was not effective to control foliar loss (FFM=902.79 mg). In comparison with C+, SHb30 tended to improve FFM (FFM=1341.0 mg) but not significantly. FFM of SHb30 was, nevertheless, not different ($p > 0.05$) from Cf and Cap controls. Once combined, FFM of SHb30+C13 increased to 1694.5 mg. This combination was different ($p \leq 0.05$) from C+ but not ($p > 0.05$) from C-. G2 and consortium containing G2 were all able to improve FFM to the same level as C- ($p \leq 0.05$) but no difference was found between them. Excepted for C13 alone, all tested treatments applied alone or in consortium gave a FFM protection as good ($p > 0.05$) as Cf and Cap controls.

CONCLUSIONS AND DISCUSSION

In this biocontrol study, it was shown that aquaponic water was able to control *P. aphanidermatum* lettuce disease. This suppressive action was already observed in Stouvenakers et al. (2020) and confirmed that aquaponics can be a source of antagonistic agents that should not be ignored for soilless use. In our study, the fungal *A. flavus* strain G2 was the best agent in sole application and was able to reduce lettuce root symptoms of *P. aphanidermatum* disease to a similar level than Cf and Cap controls. Moreover, no LM and no significant FFM loss were observed with this treatment. Although as effective as Cf and Cap, combination of G2 with C13 and/or SHb30 (both bacterial strains) did not bring significant additional effect. In relation to Stouvenakers et al. (2023), these results showed that antagonistic activity of G2 is reproducible over time. Studies on *A. flavus* use against *P. aphanidermatum* are scarce and limited to few papers (Shanmugan and Sakurana Varma, 1999; Stouvenakers et al., 2023), while atoxigenic strains were intensively studied to control aflatoxin in cereal crops (Khan et al., 2021). Activity of *S. xenophagum* strain SHb30 applied alone was less effective than G2 to control root rot but this treatment allowed to keep a FFM as good as Cf and Cap controls. Work must be undertaken to obtain stable action with SHb30 strain. *M. fortuitum* strain C13 was not able to control the disease in this test while Stouvenakers et al. (2023) reported a better efficacy. In our knowledge, this study and that of Stouvenakers et al. (2023) were the first reports of an antagonistic activity of *S. xenophagum and M. fortuitum* species against plant pathogens. Once SHb30 and C13 combined, an additional effect tended to be observed with efficacy similar to Cf and Cap controls. In literature, several strains of *P. chlororaphis*, including Tx-1 strain, were described as the most adapted biocontrol agents to control *Pythium* spp. diseases in hydroponics (Khan et al., 2003; Chatterton et al., 2004; Liu et al., 2007; Sopher and Sutton, 2011). However, its action remains variable as shown by its non-efficacy in this test and its medium efficacy reported in Stouvenakers et al. (2023). In conclusion, this study showed that aquaponics is an important source of antagonistic microorganisms that could control *P. aphanidermatum* disease on lettuce. G2 was effective to control the disease alone, while SHb30 and C13 were better in consortium.

ACKNOWLEDGEMENTS

I would like to thank my supervisors and my laboratory staff for their assistance and reviewing. I also thank the trainee Eloïse Fournier for her technical assistance.

Literature cited

Chatterton, S., Sutton, J.C., and Boland, G.J. (2004). Timing *Pseudomonas chlororaphis* applications to control *Pythium aphanidermatum*, *Pythium dissotocum*, and root rot in hydroponic peppers. Biol. Control *30* (*2*), 360–373 https://doi.org/10.1016/j.biocontrol.2003.11.001.

Khan, A., Sutton, J.C., and Grodzinski, B. (2003). Effects of *Pseudomonas chlororaphis* on *Pythium aphanidermatum* and root rot in peppers grown in small-scale hydroponic troughs. Biocontrol Sci. Technol. *13* (*6*), 615–630 https://doi.org/10.1080/0958315031000151783.

Khan, R., Ghazali, F.M., Mahyudin, N.A., and Samsudin, N.I.P. (2021). Biocontrol of aflatoxins using non-aflatoxigenic *Aspergillus flavus*: a literature review. J Fungi (Basel) *7* (*5*), 381 https://doi.org/10.3390/jof7050381. PubMed

Liu, W., Sutton, J.C., Grodzinski, B., Kloepper, J.W., and Reddy, M.S. (2007). Biological control of Pythium root rot of chrysanthemum in small-scale hydroponic units. Phytoparasitica *35* (*2*), 159–178 https://doi.org/10.1007/BF02981111.

McPherson, G.M., Harriman, M.R., and Pattison, D. (1995). The potential for spread of root diseases in recirculating hydroponic systems and their control with disinfection. Med. Fac. Landbouww. Univ. Gent *60*, 371–379.

Montagne, V., Capiaux, H., Barret, M., Cannavo, P., Charpentier, S., Grosbellet, C., and Lebeau, T. (2017). Bacterial and fungal communities vary with the type of organic substrate: implications for biocontrol of soilless crops. Environ. Chem. Lett. *15* (*3*), 537–545 https://doi.org/10.1007/s10311-017-0628-0.

Postma, J., van Os, E., and Bonants, P.J.M. (2008). Chapter 10 - Pathogen detection and management strategies in soilless plant growing system. In Soilless Culture: Theory and Practice (Elsevier BV), p.425–457.

Shanmugan, V., and Sakurana Varma, A. (1999). Effect of native antagonists against *Pythium aphanidermatum*, the causal organism of rhizome rot in ginger. J. Mycol. Plant Pathol. *29*, 375–379.

Sopher, C.R., and Sutton, J.C. (2011). Quantitative relationships of *Pseudomonas chlororaphis* 63-28 to Pythium root rot and growth in hydroponic peppers. Trop. Plant Pathol. *36* (*4*), 214–224 https://doi.org/10.1590/S1982-56762011000400002.

Stouvenakers, G., Dapprich, P., Massart, S., and Jijakli, M.H. (2019). Plant pathogens and control strategies in aquaponics. In Aquaponics Food Production Systems, Chapter 14, S. Goddek, A. Joyce, B. Kotzen, and G.M. Burnell, eds. (Cham: Springer), p.353–378.

Stouvenakers, G., Massart, S., Depireux, P., and Jijakli, M.H. (2020). Microbial origin of aquaponic water suppressiveness against *Pythium aphanidermatum* lettuce root rot disease. Microorganisms *8* (*11*), 1638 https://doi.org/10.3390/microorganisms8111683. PubMed

Stouvenakers, G., Massart, S., and Jijakli, M.H. (2023). First study case of microbial biocontrol agents isolated from aquaponics through the mining of high-throughput sequencing data to control *Pythium aphanidermatum* on lettuce. Microb Ecol *86* (*2*), 1107–1119 https://doi.org/10.1007/s00248-022-02126-1. PubMed

Sutton, J.C., Sopher, C.R., Owen-Going, T.N., Liu, W., Grodzinski, B., Hall, J.C., and Benchimol, R.L. (2006). Etiology and epidemiology of Pythium root rot in hydroponic crops: current knowledge and perspectives. Summa Phytopathol. *32* (*4*), 307–321 https://doi.org/10.1590/S0100-54052006000400001.

Vallance, J., Déniel, F., Le Floch, G., Guérin-Dubrana, L., Blancard, D., and Rey, P. (2011). Pathogenic and beneficial microorganisms in soilless cultures. Agron. Sustain. Dev. *31* (*1*), 191–203 https://doi.org/10.1051/agro/2010018.