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Aquaponics as future urban food production systems: phytopathological challenges and opportunities thanks to aquaponic microbiota characterization and original biocontrol agent isolation

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Abstract: Aquaponics is a sustainable way to produce simultaneously fish and plants, notably in urban areas. In such system, plant disease control is problematic because sanitary treatments can be toxic for fish. However, aquaponics could naturally contain microorganisms with suppressive activity against plant pathogens. Aquaponic microbial composition in the lettuce rhizosphere was studied for this activity. The high-throughput sequencing analyses highlighted numerous microorganisms that were potentially disease suppressive, including *Methyloversatilis*, *Sphingobium*, *Hydrogenophaga*, *Catenaria*, Burkholderiaceae, and Aspergillaceae taxa. After selective isolation and an *in vivo* screening step, three strains excelled in the biocontrol of diseases caused by *P. aphanidermatum* on lettuce seeds and seedlings grew in hydroponics conditions. They were SHb30 (*Sphingobium xenophagum*), G2 (*Aspergillus flavus*) and Chito13 (*Mycolicibacterium fortuitum*). Their combinations were also efficient in controlling lettuce root rot disease caused by the same pathogen. This study showed that aquaponics is an interesting source of original biocontrol agents that could be used in soilless conditions to improve plant health and then urban food supply.

Key words: aquaponics, hydroponics, biocontrol, antagonist, consortium, urban

Introduction

Aquaponics – an integrated way to produce fish and hydroponic plants in the same production system – was emphasised as a sustainable issue of urban food requirement concerns (Goddek et al., 2015). Among the challenges in developing aquaponic food production system, managing plant diseases is a prior question (Goddek et al., 2015; Stouvenakers et al., 2019). Indeed, using phytosanitary treatments to control soil-borne diseases is not recommended in environments where fish and plants share the same irrigation water (Stouvenakers et al., 2019). Mainly, pesticides or chemical disinfection agents to control root pathogens are inadvisable because of their toxicity to fish and beneficial microorganisms in the system (Rakocy et al., 2006; Nemethy et al., 2016; Stouvenakers et al., 2019). In aquaponics, lettuce is one of the main valuable and cultivated crops (Bailey and Ferrarezi, 2017), while root pathogens, and more significantly, oomycetes pathogens able to produce mobile forms of dispersion, are particularly problematic for soilless lettuce cultivation. Among oomycetes, *Pythium aphanidermatum* (Edson) Fitzp. is one of the most damaging lettuce root pathogens in hydroponic conditions (Utkhede et al., 2000; Postma et al., 2008). However, it was emphasised in Stouvenakers et al., 2019, and later showed in Stouvenakers et al., 2020, that an aquaponic system could be naturally plant pathogens suppressive by the involvement of antagonistic microorganisms. This study gathers the results from the characterization of a plant pathogens suppressive aquaponic microbiota and the arising steps of biocontrol agent isolation and efficacy validation.

Materials and methods

Root microbial communities of lettuce grew in aquaponic water were analysed by high-throughput sequencing (HTS) to determine the origin of the aquaponic suppressiveness observed against *P. aphanidermatum* (see Stouvenakers et al., 2020). The bacterial and fungal composition of aquaponic lettuce rhizoplane was compared with that of lettuce that grew in a *P. aphanidermatum* conducive hydroponic water. Correlations and differential analyses were then conducted to identify microbial taxa involved in disease suppression (see Stouvenakers et al., 2020). Microorganisms identified as potentially involved in disease suppression were tried to be selectively isolated with a HTS-guided approach (see Stouvenakers et al., 2022). Isolated strains of bacteria and fungi were then screened *in vivo* to control lettuce seed damping-off caused by *P. aphanidermatum* (see Stouvenakers et al., 2022). Most promising strains were individually tested in hydroponic conditions to control *P. aphanidermatum* root rot on lettuce seedlings (see Stouvenakers et al., 2022). In a similar experiment, the three most efficacious strains were tested in different consortium combinations (see Stouvenakers et al., 2023).

Results

Microbial β-diversity was different ($p \leq 0.05$) between aquaponic and hydroponic lettuce rhizoplane. Moreover, microbial diversity (Shannon index) was also higher ($p \leq 0.05$) in aquaponics. Statistical comparison in microbial taxa composition highlighted a list of bacteria and fungi correlated to disease suppression in aquaponics (see Stouvenakers et al., 2020). Among this list of potential biocontrol agents, the taxa belonging to the genera *Methyloversatilis*, *Sphingobium*, *Hydrogenophaga* and *Catenaria*, and the family Burkholderiaceae, and Aspergillaceae were chosen for selective isolation. One hundred strains were isolated and screened *in vivo* to control lettuce seed damping-off caused by *P. aphanidermatum*. Out of these isolates, 27 strains, distributed in seven species of bacteria and one species of fungi, were determined efficacious in controlling lettuce seed damping-off caused by *P. aphanidermatum* (see Stouvenakers et al., 2022). Out of these eight species, three species, each represented by a selected strain, showed better abilities to decrease symptoms caused by *P. aphanidermatum* in lettuce seedlings (Figure 1) (see Stouvenakers et al., 2022). These strains were SHb30 (*Sphingobium xenophagum*), G2 (*Aspergillus flavus*) and Chito13 (*Mycolicibacterium fortuitum*). In brief, seed damping-off was decreased following the application of the three strains and at a better rate than a propamocarb fungicide control (Cf) and a *Pseudomonas chlororaphis* registered biocontrol agent control (Cpc) did. Mortality caused by the pathogen at the seedling stage was also prevented by the application of the three strains and their later combination. Root rot symptoms were reduced ($p \leq 0.05$) – at least as good as the two controls – by the application of the three strains (Figure 2 a) and then their consortia (Figure 2 b).

Figure 1. Pictural representation of disease symptoms caused by *P. aphanidermatum* on lettuce seedlings following treatment with Chito13, SHb30, and G2 microbial strains. C+ and C- are the positive (inoculated) and the negative (non-inoculated) control, respectively.

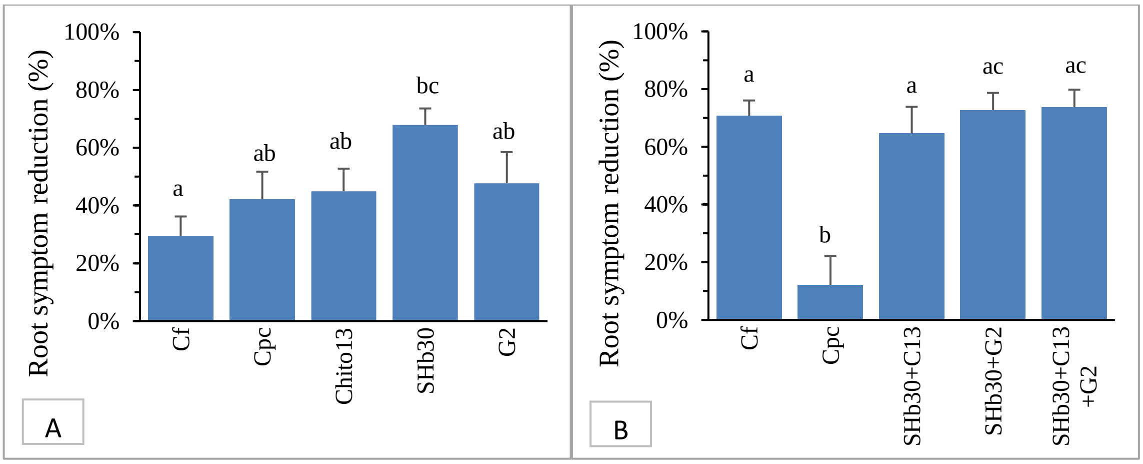

Figure 2. Mean bar charts of root rot symptom reduction following treatment with Chito13, SHb30, and G2 microbial strains applied individually (A) or in consortia (B) to control *P. aphanidermatum* disease on lettuce seedlings (Stouvenakers et al., 2022, 2023). Cf and Cpc are the propamocarb fungicide control, and the *Pseudomonas chlororaphis* registered biocontrol agent control, respectively. Bars indicate the standard error of the mean. Different letters indicate significant differences ($p \leq 0.05$) between treatments according to Tukey's ANOVA *post hoc* test.

Discussion and conclusion

Results gathered from our studies highlighted that aquaponic water naturally hosted pathogens suppressive microorganisms. Microorganisms that were original considering the literature on microbial biocontrol agents in plants diseases management. After selective isolation, seven bacterial and one fungal species could control lettuce seed damping-off caused by *P. aphanidermatum*. Out of these eight microbial species, three species strains were particularly efficacient in controlling *P. aphanidermatum* root rot disease on lettuce seedlings. They were SHb30 (*Sphingobium xenophagum*), G2 (*Aspergillus flavus*) and Chito13 (*Mycolicibacterium fortuitum*). Their combinations were also efficient in controlling the disease, but the adding value of consortia application must be further studied. While not observed in our studies, *A. flavus* and *M. fortuitum* were reported as potential plant and fish pathogens, respectively (Amaike and Keller, 2011; Fattah and Sayed, 2006). Consequently, SHb30 is considered as the most promising strain for aquaponic use. Moreover, the species was described as a potential plant biostimulant (Wanees et al., 2018) and an agent able to play a role in the bioremediation of contaminated environments (Song et al., 2019).

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