# **Chapter 14 Plant Pathogens and Control Strategies in Aquaponics**



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**Abstract** Among the diversity of plant diseases occurring in aquaponics, soil-borne pathogens, such as *Fusarium* spp., *Phytophthora* spp. and *Pythium* spp., are the most problematic due to their preference for humid/aquatic environment conditions. Phytophthora spp. and Pythium spp. which belong to the Oomycetes pseudo-fungi require special attention because of their mobile form of dispersion, the so-called zoospores that can move freely and actively in liquid water. In coupled aquaponics, curative methods are still limited because of the possible toxicity of pesticides and chemical agents for fish and beneficial bacteria (e.g. nitrifying bacteria of the biofilter). Furthermore, the development of biocontrol agents for aquaponic use is still at its beginning. Consequently, ways to control the initial infection and the progression of a disease are mainly based on preventive actions and water physical treatments. However, suppressive action (suppression) could happen in aquaponic environment considering recent papers and the suppressive activity already highlighted in hydroponics. In addition, aquaponic water contains organic matter that could promote establishment and growth of heterotrophic bacteria in the system or even improve plant growth and viability directly. With regards to organic hydroponics (i.e. use of organic fertilisation and organic plant media), these bacteria could act as antagonist agents or as plant defence elicitors to protect plants from diseases. In the future, research on the disease suppressive ability of the aquaponic biotope must be increased, as well as isolation, characterisation and formulation of microbial plant pathogen antagonists. Finally, a good knowledge in the rapid identification of pathogens, combined with control methods and diseases monitoring, as recommended in integrated plant pest management, is the key to an efficient control of plant diseases in aquaponics.

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## 14.1 Introduction

Nowadays, aquaponic systems are the core of numerous research efforts which aim at better understanding these systems and at responding to new challenges of food production sustainability (Goddek et al. 2015; Villarroel et al. 2016). The cumulated number of publications mentioning "aquaponics" or derived terms in the title went from 12 in early 2008 to 215 in 2018 (January 2018 Scopus database research results). In spite of this increasing number of papers and the large area of study topics they are covering, one critical point is still missing, namely plant pest management (Stouvenakers et al. 2017). According to a survey on EU Aquaponic Hub members, only 40% of practitioners have some notions about pests and plant pest control (Villarroel et al. 2016).

In aquaponics, the diseases might be similar to those found in hydroponic systems under greenhouse structures. Among the most problematic pathogens, in term of spread, are hydrophilic fungi or fungus-like protists which are responsible for root or collar diseases. To consider plant pathogen control in aquaponics, firstly, it is important to differentiate between coupled and decoupled systems. Decoupled systems allow disconnection between water from the fish and crop compartment (see Chap. 8). This separation allows the optimisation and a better control of different parameters (e.g. temperature, mineral or organic composition and pH) in each compartment (Goddek et al. 2016; Monsees et al. 2017). Furthermore, if the water from the crop unit does not come back to the fish part, the application of phytosanitary treatments (e.g. pesticides, biopesticides and chemical disinfection agents) could be allowed here. Coupled systems are built in one loop where water recirculates in all parts of the system (see Chaps. 5 and 7). However, in coupled systems, plant pest control is more difficult due to the both presence of fish and beneficial microorganisms which transform fish sludge into plant nutrients. Their existence limits or excludes the application of already available disinfecting agents and chemical treatments. Furthermore no pesticides or biopesticides have been specifically developed for aquaponics (Rakocy et al. 2006; Rakocy 2012; Somerville et al. 2014; Bittsanszky et al. 2015; Nemethy et al. 2016; Sirakov et al. 2016). Control measures are consequently mainly based on non-curative physical practices (see Sect. 14.3.1) (Nemethy et al. 2016; Stouvenakers et al. 2017).

On the other hand, recent studies highlight that aquaponic plant production offers similar yields when compared to hydroponics although concentrations of mineral plant nutrients are lower in aquaponic water. Furthermore, when aquaponic water is complemented with some minerals to reach hydroponic concentrations of mineral nutritive elements, even better yields can be observed (Pantanella et al. 2010; Pantanella et al. 2015; Delaide et al. 2016; Saha et al. 2016; Anderson et al. 2017;

Wielgosz et al. 2017; Goddek and Vermeulen 2018). Moreover, some informal observations from practitioners in aquaponics and two recent scientific studies (Gravel et al. 2015; Sirakov et al. 2016) report the possible presence of beneficial compounds and/or microorganisms in the water that could play a role in biostimulation and/or have antagonistic (i.e. inhibitory) activity against plant pathogens. Biostimulation is defined as the enhancement of plant quality traits and plant tolerance against abiotic stress using any microorganism or substance.

With regard to these aspects, this chapter has two main objectives. The first is to give a review of microorganisms involved in aquaponic systems with a special focus on plant pathogenic and plant beneficial microorganisms. Factors influencing these microorganisms will be also considered (e.g. organic matter). The second is to review available methods and future possibilities in plant diseases control.

#### 14.2 Microorganisms in Aquaponics

Microorganisms are present in the entire aquaponics system and play a key role in the system. They are consequently found in the fish, the filtration (mechanical and biological) and the crop parts. Commonly, the characterisation of microbiota (i.e. microorganisms of a particular environment) is carried out on circulating water, periphyton, plants (rhizosphere, phyllosphere and fruit surface), biofilter, fish feed, fish gut and fish faeces. Up until now, in aquaponics, most of microbial research has focused on nitrifying bacteria (Schmautz et al. 2017). Thus, the trend at present is to characterise microorganisms in all compartments of the system using modern sequencing technologies. Schmautz et al. (2017) identified the microbial composition in different parts of the system, whereas Munguia-Fragozo et al. (2015) give perspectives on how to characterize aquaponics microbiota from a taxonomical and functional point of view by using cutting-edge technologies. In the following sub-sections, focus will be only brought on microorganisms interacting with plants in aquaponic systems organised into plant beneficial and plant pathogenic microorganisms.

#### 14.2.1 Plant Pathogens

Plant pathogens occurring in aquaponic systems are theoretically those commonly found in soilless systems. A specificity of aquaponic and hydroponic plant culture is the continuous presence of water in the system. This humid/aquatic environment suits almost every plant pathogenic fungus or bacteria. For root pathogens some are particularly well adapted to these conditions like pseudo-fungi belonging to the taxa of Oomycetes (e.g. root rot diseases caused by *Pythium* spp. and *Phytophthora* spp.) which are able to produce a motile form of dissemination called zoospores. These zoospores are able to move actively in liquid water and thus are able to spread over

the entire system extremely quickly. Once a plant is infected, the disease can rapidly spread out the system, especially because of the water's recirculation (Jarvis 1992; Hong and Moorman 2005; Sutton et al. 2006; Postma et al. 2008; Vallance et al. 2010; Rakocy 2012; Rosberg 2014; Somerville et al. 2014). Though Oomycetes are among the most prevalent pathogens detected during root diseases, they often form a complex with other pathogens. Some Fusarium species (with existence of species well adapted to aquatic environment) or species from the genera Colletotrichum, Rhizoctonia and Thielaviopsis can be found as part of these complexes and can also cause significant damage on their own (Paulitz and Bélanger 2001; Hong and Moorman 2005; Postma et al. 2008; Vallance et al. 2010). Other fungal genera like Verticillium and Didymella, but also bacteria, such as Ralstonia, Xanthomonas, Clavibacter, Erwinia and Pseudomonas, as well as viruses (e.g. tomato mosaic, cucumber mosaic, melon necrotic spot virus, lettuce infectious virus and tobacco necrosis), can be detected in hydroponics or irrigation water and cause vessel, stem. leaf or fruit damage (Jarvis 1992; Hong and Moorman 2005). However note that not all microorganisms detected are damaging or lead to symptoms in the crop. Even species of the same genus can be either harmful or beneficial (e.g. Fusarium, Phoma, Pseudomonas). Disease agents discussed above are mainly pathogens linked to water recirculation but can be identified in greenhouses also. Section 14.2.2 shows the results of the first international survey on plant diseases occurring specifically in aquaponics, while Jarvis (1992) and Albajes et al. (2002) give a broader view of occurring pathogens in greenhouse structures.

In hydroponics or in aquaponic systems, plants generally grow under greenhouse conditions optimized for plant production, especially for large-scale production where all the environmental parameters are computer managed (Albajes et al. 2002; Vallance et al. 2010; Somerville et al. 2014; Parvatha Reddy 2016). However, optimal conditions for plant production can also be exploited by plant pathogens. In fact, these structures generate warm, humid, windless and rain-free conditions that can encourage plant diseases if they are not correctly managed (ibid.). To counteract this, compromises must be made between optimal plant conditions and disease prevention (ibid.). In the microclimate of the greenhouse, an inappropriate management of the vapour-pressure deficit can lead to the formation of a film or a drop of water on the plants surface. This often promotes plant pathogen development. Moreover, to maximise the yield in commercial hydroponics, some other parameters (e.g. high plant density, high fertilisation, to extend the period production) can enhance the susceptibility of plants to develop diseases (ibid.).

The question now is to know by which route the initial inoculum (i.e. the first step in an epidemiological cycle) is brought into the system. The different steps in plant disease epidemiological cycle (EpC) are represented in Fig. 14.1. In aquaponics, as in greenhouse hydroponic culture, it can be considered that entry of pathogens could be linked to water supply, introduction of infected plants or seeds, the growth material (e.g. reuse of the media), air exchange (dust and particles carriage), insects (vectors of diseases and particles carriage) and staff (tools and clothing) (Paulitz and Bélanger 2001; Albajes et al. 2002; Hong and Moorman 2005; Sutton et al. 2006; Parvatha Reddy 2016).



**Fig. 14.1** Basic steps (*1* to 6) in plant disease epidemiological cycle (EpC) according to Lepoivre (2003). (*1*) Arrival of the pathogen inoculum, (2) contact with the host plant, (3) tissues penetration and infection process by the pathogen, (4) symptoms development, (5) plant tissues that become infectious, (6) release and spread of infectious form of dispersion

Once the inoculum is in contact with the plant (step 2 in the EpC), several cases of infection (step 3 in the EpC) are possible (Lepoivre 2003):

- The pathogen-plant relationship is incompatible (non-host relation) and disease does not develop.
- There is a host relation but the plant does not show symptoms (the plant is tolerant).
- The pathogen and the plant are compatible but defence response is strong enough to inhibit the progression of the disease (the plant is resistant: interaction between host resistance gene and pathogen avirulence gene).
- The plant is sensitive (host relation without gene for gene recognition), and the pathogen infects the plant, but symptoms are not highly severe (step 4 in the EpC).
- And lastly, the plant is sensitive and disease symptoms are visible and severe (step 4 in the EpC).

Regardless of the degree of resistance, some environmental conditions or factors can influence the susceptibility of a plant to be infected, either by a weakening of the plant or by promoting the growth of the plant pathogen (Colhoun 1973; Jarvis 1992; Cherif et al. 1997; Alhussaen 2006; Somerville et al. 2014). The main environmental factors influencing plant pathogens and disease development are temperature, relative humidity (RH) and light (ibid.). In hydroponics, temperature and oxygen concentrations within the nutrient solution can constitute additional factors (Cherif et al. 1997; Alhussaen 2006; Somerville et al. 2014). Each pathogen has its own preference of environmental conditions which can vary during its epidemiologic cycle. But in a general way, high humidity and temperature are favourable to the accomplishment of key steps in the pathogen's epidemic cycle such as spore production or spore germination (Fig. 14.1, step 5 in the EpC) (Colhoun 1973; Jarvis 1992; Cherif et al. 1997; Alhussaen 2006; Somerville et al. 2014). Colhoun (1973) sums up the effects of the various factors promoting plant diseases in soil, whereas Table 14.1 shows the more specific or adding factors that may encourage plant pathogen development linked to aquaponic greenhouse conditions.

In the epidemiological cycle, once the infective stage is reached (step 5 in the EpC), the pathogens can spread in several ways (Fig. 14.1, step 6 in the EpC) and infect other plants. As explained before, root pathogens belonging to Oomycetes taxa can actively spread in the recirculating water by zoospores release (Alhussaen 2006; Sutton et al. 2006). For other fungi, bacteria and viruses responsible for root or aerial diseases, the dispersion of the causal agent can occur by propagation of infected material, mechanical wounds, infected tools, vectors (e.g. insects) and particles (e.g. spores and propagules) ejection or carriage allowed by drought, draughts or water splashes (Albajes et al. 2002; Lepoivre 2003).

#### 14.2.2 Survey on Aquaponic Plant Diseases

During January 2018, the first international survey on plant diseases was made among aquaponics practitioner members of the COST FA1305, the American Aquaponics Association and the EU Aquaponics Hub. Twenty-eight answers were

Promoting factor	Profiting to	Causes	References
Nutrient film tech- nique (NFT), Deep flow technique (DFT)	Pythium spp., Fusarium spp.	Easy spread by water recirculation; possibility of post contamination after a disinfection step; poor content in oxygen in the nutrient solution	Koohakan et al. (2004) and Vallance et al. (2010)
Inorganic media (e.g. rockwool)	Higher content in bacteria (no information about their possible pathogenicity)	Unavailable organic compounds in the media	Khalil and Alsanius (2001), Koohakan et al. (2004), Vallance et al. (2010)
Organic media (e.g. coconut fibre and peat)	Higher content in fungi; higher con- tent in <i>Fusarium</i> spp. for coconut fibre	Available organic com- pounds in the media	Koohakan et al. (2004), Khalil et al. (2009), and Vallance et al. (2010)
Media with high water content and low content in oxygen (e.g. rockwool)	Pythium spp.	Zoospores mobility; plant stress	Van Der Gaag and Wever (2005), Vallance et al. (2010), and Khalil and Alsanius (2011)
Media allowing lit- tle water move- ment (e.g. rockwool)	Pythium spp.	Better condition for zoo- spores dispersal and che- motaxis movement; no loss of zoospore flagella	Sutton et al. (2006)
High temperature and low concentra- tion of DO in the nutrient solution	Pythium spp.	Plant stressed and optimal condition for <i>Pythium</i> growth	Cherif et al. (1997), Sutton et al. (2006), Vallance et al. (2010), and Rosberg (2014)
High host plant density and resulting microclimate	Pathogens growth; diseases spread	Warm and humid environment	Albajes et al. (2002) and Somerville et al. (2014)
Deficiencies, excess or imbal- ance of macro/ micronutrients	Fungi, viruses and bacteria	Plant physiological mod- ifications (e.g. action on defence response, tran- spiration, integrity of cell walls); plant morphologi- cal modifications (e.g. higher susceptibility to pathogens, attraction of pests); nutrient resources in host tissues for patho- gens; direct action on the pathogen development cycle	Colhoun (1973), Snoeijers and Alejandro (2000), Mitchell et al. (2003), Dordas (2008), Veresoglou et al. (2013), Somerville et al. (2014), and Geary et al. (2015)

 Table 14.1
 Adding factors encouraging plant pathogen development under aquaponic greenhouse structure compared to classical greenhouse culture

received describing 32 aquaponic systems from around the world (EU, 21; North America, 5; South America, 1; Africa, 4; Asia, 1). The first finding was the small response rate. Among the possible explanations for the reluctance to reply to the questionnaire was that practitioners did not feel able to communicate about plant pathogens because of a lack of knowledge on this topic. This had already been observed in the surveys of Love et al. (2015) and Villarroel et al. (2016).

Key information obtained from the survey are:

- 84.4% of practitioners observe disease in their system.
- 78.1% cannot identify the causal agent of a disease.
- 34.4% do not apply disease control measures.
- 34.4% use physical or chemical water treatment.
- 6.2% use pesticides or biopesticides in coupled aquaponic system against plant pathogens.

These results support the previous arguments saying that aquaponic plants do get diseases. Yet, practitioners suffer from a lack of knowledge about plant pathogens and disease control measures actually used are essentially based on non-curative actions (90.5% of cases).

In the survey, a listing of plant pathogens occurring in their aquaponic system was provided. Table 14.2 shows the results of this identification. To remedy the lack of practitioner's expertise about plant disease diagnostics, a second survey version was

Plant host	Plant pathogen	References or survey results
Allium schoenoprasu	<i>Pythium</i> sp. <sup>(b)</sup>	Survey
Beta vulgaris (swiss chard)	Erysiphe betae <sup>(a)</sup>	Survey
Cucumis sativus	Podosphaera xanthii <sup>(a)</sup>	Survey
Fragaria spp.	Botrytis cinerea <sup>(a)</sup>	Survey
Lactuca sativa	Botrytis cinerea <sup>(a)</sup>	Survey
	Bremia lactucae <sup>(a)</sup>	Survey
	Fusarium sp. <sup>(b)</sup>	Survey
	Pythium dissotocum <sup>(b)</sup>	Rakocy (2012)
	Pythium myriotylum <sup>(b)</sup>	Rakocy (2012)
	Sclerotinia sp. <sup>(a)</sup>	Survey
Mentha spp.	<i>Pythium</i> sp. <sup>(b)</sup>	Survey
Nasturtium officinale	Aspergillus sp. <sup>(a)</sup>	Survey
Ocimum basilicum	Alternaria sp. <sup>(a)</sup>	Survey
	Botrytis cinerea <sup>(a)</sup>	Survey
	<i>Pythium</i> sp. <sup>(b)</sup>	Survey
	Sclerotinia sp. <sup>(a)</sup>	Survey
Pisum sativum	Erysiphe pisi <sup>(a)</sup>	Survey
Solanum lycopersicum	Pseudomononas solanacearum <sup>(a)</sup>	McMurty et al. (1990)
	Phytophthora infestans <sup>(a)</sup>	Survey

**Table 14.2** Results of the first identifications of plant pathogens in aquaponics from the 2018 international survey analysis and from existing literature

Plant pathogens identified by symptoms in the aerial plant part are annotated by (a) and in root part by (b) in exponent

sent with the aim to identify symptoms without disease name linkage (Table 14.3). Table 14.2 mainly identifies diseases with specific symptoms, i.e. symptoms that can be directly linked to a plant pathogen. It is the case of *Botrytis cinerea* and its typical grey mould, powdery mildew (Erysiphe and Podosphaera genera in the table) and its white powdery mycelium/conidia, and lastly *Sclerotinia* spp. and its sclerotia production. The presence of 3 plant pathologists in the survey respondents expands the list, with the identification of some root pathogens (e.g. Pythium spp.). General symptoms that are not specific enough to be directly related to a pathogen without further verification (see diagnosis in Sect. 14.3) are consequently found in Table 14.3. But it is important to highlight that most of the symptoms observed in this table could also be the consequence of abiotic stresses. Foliar chlorosis is one of the most explicit examples because it can be related to a large number of pathogens (e.g. for lettuces: Pythium spp., Bremia lactucae, Sclerotinia spp., beet western yellows virus), to environmental conditions (e.g. temperature excess) and to mineral deficiencies (nitrogen, magnesium, potassium, calcium, sulfur, iron, copper, boron, zinc, molybdenum) (Lepoivre 2003; Resh 2013).

Symptoms	Plants species
Foliar chlorosis	Allium schoenoprasum <sup>1</sup> , Amaranthus viridis <sup>1</sup> , Coriandrum sativum <sup>1</sup> , Cucumis sativus <sup>1</sup> , Ocimum basilicum <sup>6</sup> , Lactuca sativa <sup>4</sup> , Mentha spp. <sup>2</sup> , Petroselinum crispum <sup>1</sup> , Spinacia oleracea <sup>2</sup> , Solanum lycopersicum <sup>1</sup> , Fragaria spp. <sup>1</sup>
Foliar necrosis	Mentha spp. <sup>2</sup> , Ocimum basilicum <sup>1</sup> ,
Stem necrosis	Solanum lycopersicum <sup>1</sup> ,
Collar necrosis	Ocimum basilicum <sup>1</sup>
Foliar Mosaic	<i>Cucumis sativus</i> <sup>1</sup> , <i>Mentha</i> spp. <sup>1</sup> , <i>Ocimum basilicum</i> <sup>1</sup> ,
Foliar wilting	Brassica oleracea Acephala group <sup>1</sup> , Lactuca sativa <sup>1</sup> , Mentha spp. <sup>1</sup> , Cucumis sativus <sup>1</sup> , Ocimum basilicum <sup>1</sup> , Solanum lycopersicum <sup>1</sup>
Foliar, stem and collar mould	Allium schoenoprasum <sup>1</sup> , Capsicum annuum <sup>1</sup> , Cucumis sativus <sup>1</sup> , Lactuca sativa <sup>2</sup> , Mentha spp. <sup>2</sup> , Ocimum basilicum <sup>4</sup> , Solanum lycopersicum <sup>1</sup>
Foliar spots	<i>Capsicum annuum</i> <sup>1</sup> , <i>Cucumis sativus</i> <sup>1</sup> , <i>Lactuca sativa</i> <sup>2</sup> , <i>Mentha spp.</i> <sup>1</sup> , <i>Ocimum basilicum</i> <sup>5</sup>
Damping off	Spinacia oleracea <sup>1</sup> , Ocimum basilicum <sup>1</sup> , Solanum lycopersicum <sup>1</sup> , seedlings in general <sup>5</sup>
Crinkle	<i>Beta vulgaris</i> (swiss chard) <sup>1</sup> , <i>Capsicum annuum</i> <sup>1</sup> , <i>Lactuca sativa</i> <sup>1</sup> , <i>Ocimum basilicum</i> <sup>1</sup>
Browning or decaying root	Allium schoenoprasum <sup>1</sup> , Amaranthus viridis <sup>1</sup> , Beta vulgaris (swiss chard) <sup>1</sup> , Coriandrum sativum <sup>1</sup> , Lactuca sativa <sup>1</sup> , Mentha spp. <sup>2</sup> , Ocimum basilicum <sup>2</sup> , Petroselinum crispum <sup>2</sup> , Solanum lycopersicum <sup>1</sup> , Spinacia oleracea <sup>1</sup>

 Table 14.3 Review of occurring symptoms in aquaponics from the 2018 international survey analysis

Numbers in exponent represent the occurrence of the symptom for a specific plant on a total of 32 aquaponic systems reviewed

# 14.2.3 Beneficial Microorganisms in Aquaponics: The Possibilities

As explained in the introduction, several publications focused on bacteria involved in the nitrogen cycle, while others already emphasise the potential presence of beneficial microorganisms interacting with plant pathogens and/or plants (Rakocy 2012; Gravel et al. 2015; Sirakov et al. 2016). This section reviews the potential of plant beneficial microorganisms involved in aquaponics and their modes of action.

Sirakov et al. (2016) screened antagonistic bacteria against Pythium ultimum isolated from an aquaponic system. Among the 964 tested isolates, 86 showed a strong inhibitory effect on Pythium ultimum in vitro. Further research must be achieved to taxonomically identify these bacteria and evaluate their potential in in vivo conditions. The authors assume that many of these isolates belong to the genus Pseudomonas. Schmautz et al. (2017) came to the same conclusion by identifying *Pseudomonas* spp. in the rhizosphere of lettuce. Antagonistic species of the genus Pseudomonas were able to control plant pathogens in natural environments (e.g. in suppressive soils) while this action is also affected by environmental conditions. They can protect plants against pathogens either in an active or a passive way by eliciting a plant defence response, playing a role in plant growth promotion, compete with pathogens for space and nutrients (e.g. iron competition by release of iron-chelating siderophores), and/or finally by production of antibiotics or antifungal metabolites such as biosurfactants (Arras and Arru 1997; Ganeshan and Kumar 2005; Haas and Défago 2005; Beneduzi et al. 2012; Narayanasamy 2013). Although no identification of microorganisms was done by Gravel et al. (2015)), they report that fish effluents have the capacity to stimulate plant growth, decrease the mycelial growth of Pythium ultimum and Pythium oxysporum in vitro and reduce the colonization of tomato root by these fungi.

Information about the possible natural plant protection capacity of aquaponic microbiota is scarce, but the potential of this protective action can be envisaged with regard to different elements already known in hydroponics or in recirculated aquaculture. A first study was conducted in 1995 on suppressive action or suppressiveness promoted by microorganisms in soilless culture (McPherson et al. 1995). Suppressiveness in hydroponics, here defined by Postma et al. (2008)), has been "referred to the cases where (i) the pathogen does not establish or persist; or (ii) establishes but causes little or no damage". The suppressive action of a milieu can be related to the abiotic environment (e.g. pH and organic matter). However, in most situations, it is considered to be related directly or indirectly to microorganisms' activity or their metabolites (James and Becker 2007). In soilless culture, the suppressive capacity shown by water solution or the soilless media is reviewed by Postma et al. (2008) and Vallance et al. (2010). In these reviews, microorganisms responsible for this suppressive action are not clearly identified. In contrast, plant pathogens like Phytophthora cryptogea, Pythium spp., Pythium aphanidermatum and Fusarium oxysporum f.sp. radicis-lycopersici controlled or suppressed by the natural microbiota are exhaustively described. In the various articles reviewed by

Postma et al. (2008) and Vallance et al. (2010), microbial involvement in the suppressive effect is generally verified via a destruction of the microbiota of the soilless substrate by sterilisation first and eventually followed by a re-inoculation. When compared with an open system without recirculation, suppressive activity in soilless systems could be explained by the water recirculation (McPherson et al. 1995; Tu et al. 1999, cited by Postma et al. 2008) which could allow a better development and spread of beneficial microorganisms (Vallance et al. 2010).

Since 2010, suppressiveness of hydroponic systems has been generally accepted and research topics have been more driven on isolation and characterization of antagonistic strains in soilless culture with *Pseudomonas* species as main organisms studied. If it was demonstrated that soilless culture systems can offer suppressive capacity, there is no similar demonstration of such activity in aquaponics systems. However, there is no empiric indication that it should not be the case. This optimism arises from the discoveries of Gravel et al. (2015) and Sirakov et al. (2016) described in the second paragraph of this section. Moreover, it has been shown in hydroponics (Haarhoff and Cleasby 1991 cited by Calvo-bado et al. 2003; Van Os et al. 1999) but also in water treatment for human consumption (reviewed by Verma et al. 2017) that slow filtration (described in Sect. 14.3.1) and more precisely slow sand filtration can also act against plant pathogens by a microbial suppressive action in addition to other physical factors. In hydroponics, slow filtration has been demonstrated to be effective against the plant pathogens reviewed in Table 14.4. It is assumed that the microbial suppressive activity in the filters is most probably due to species of Bacillus and/or Pseudomonas (Brand 2001; Déniel et al. 2004; Renault et al. 2007; Renault et al. 2012). The results of Déniel et al. (2004) suggest that in hydroponics, the mode of action of Pseudomonas and Bacillus relies on competition for nutrients and antibiosis, respectively. However, additional modes of action could be present for these two genera as already explained for *Pseudomonas* spp. *Bacillus* species can, depending on the environment, act either indirectly by plant biostimulation or elicitation of plant defences or directly by antagonism via production of antifungal and/or antibacterial substances. Cell wall-degrading enzymes, bacteriocins, and antibiotics, lipopeptides (i.e. biosurfactants), are identified as key molecules for the latter action (Pérez-García et al. 2011; Beneduzi et al. 2012;

Plant pathogens	References
Xanthomonas campestris	Brand (2001)
pv. Pelargonii	
Fusarium oxysporum	Wohanka (1995), Ehret et al. (1999) cited by Ehret et al. (2001),
	van Os et al. (2001), Déniel et al. (2004), and Furtner et al. (2007)
Pythium spp.	Déniel et al. (2004)
Pythium aphanidermatum	Ehret et al. (1999) cited by Ehret et al. (2001), and Furtner et al. (2007)
Phytophthora cinnamomi	Van Os et al. (1999), 4 references cited by Ehret et al. (2001)
Phytophthora cryptogea	Calvo-bado et al. (2003)
Phytophthora cactorum	Evenhuis et al. (2014)

Table 14.4 Review of plant pathogens effectively removed by slow filtration in hydroponics

Narayanasamy 2013). All things considered, the functioning of a slow filter is not so different from the functioning of some biofilters used in aquaponics. Furthermore, some heterotrophic bacteria like *Pseudomonas* spp. were already identified in aquaponics biofilters (Schmautz et al. 2017). This is in accordance with the results of other researchers who frequently detected *Bacillus* and/or *Pseudomonas* in RAS (recirculated aquaculture system) biofilters (Tal et al. 2003; Sugita et al. 2005; Schreier et al. 2010; Munguia-Fragozo et al. 2015; Rurangwa and Verdegem 2015). Nevertheless, up until now, no study about the possible suppressiveness in aquaponic biofilters has been carried out.

## 14.3 Protecting Plants from Pathogens in Aquaponics

At the moment aquaponic practitioners operating a coupled system are relatively helpless against plant diseases when they occur, especially in the case of root pathogens. No pesticide nor biopesticide is specifically developed for aquaponic use (Rakocy 2007; Rakocy 2012; Somerville et al. 2014; Bittsanszky et al. 2015; Sirakov et al. 2016). In brief, curative methods are still lacking. Only Somerville et al. (2014) list the inorganic compounds that may be used against fungi in aquaponics. In any case, an appropriate diagnostic of the pathogen(s) causing the disease is mandatory in order to identify the target(s) for curative measures. This diagnosis requires good expertise in terms of observation capacity, plant pathogen cycle understanding and analysis of the situation. However, in case of generalist (not specific) symptoms and depending on the degree of accuracy needed, it is often necessary to use laboratory techniques to validate the hypothesis with respect to the causal agent (Lepoivre 2003). Postma et al. (2008) reviewed the different methods to detect plant pathogens in hydroponics, and four groups were identified:

- 1. Direct macroscopic and microscopic observation of the pathogen
- 2. Isolation of the pathogen
- 3. Use of serological methods
- 4. Use of molecular methods

#### 14.3.1 Non-biological Methods of Protection

Good agricultural practices (GAP) for plant pathogens control are the various actions aiming to limit crop diseases for both yield and quality of produce (FAO 2008). GAP transposable to aquaponics are essentially non-curative physical or cultivation practices that can be divided in preventive measures and water treatment.

#### **Preventive Measures**

Preventive measures have two distinct purposes. The first is to avoid the entry of the pathogen inoculum into the system and the second is to limit (i) plant infection,

(ii) development and (iii) spread of the pathogen during the growing period. Preventive measures aiming to avoid the entry of the initial inoculum in the greenhouse are, for example, a fallow period, a specific room for sanitation, room sanitation (e.g. plant debris removal and surface disinfection), specific clothes, certified seeds, a specific room for plant germination and physical barriers (against insect vectors) (Stanghellini and Rasmussen 1994; Jarvis 1992; Albajes et al. 2002; Somerville et al. 2014; Parvatha Reddy 2016). Among the most important practices used for the second type of preventive measures are, the use of resistant plant varieties, tools disinfection, avoidance of plant abiotic stresses, good plant spacing, avoidance of algae development and environmental conditions management. The last measure, i.e. environmental conditions management, means to control all greenhouse parameters in order to avoid or limit diseases by intervening in their biological cycle (ibid.). Generally, in large-scale greenhouse structures, computer software and algorithms are used to calculate the optimal parameters allowing both plant production and disease control. The parameters measured, among others, are temperature (of the air and the nutrient solution), humidity, vapour pressure deficit, wind speed, dew probability, leaf wetness and ventilation (ibid.). The practitioner acts on these parameters by manipulating the heating, the ventilation, the shading, the supplement of lights, the cooling and the fogging (ibid.).

#### Water Treatment

Physical water treatments can be employed to control potential water pathogens. Filtration (pore size less than 10 µm), heat and UV treatments are among the most effective to eliminate pathogens without harmful effects on fish and plant health (Ehret et al. 2001; Hong and Moorman 2005; Postma et al. 2008; Van Os 2009; Timmons and Ebeling 2010). These techniques allow the control of disease outbreaks by decreasing the inoculum, the quantity of pathogens and their proliferation stages in the irrigation system (ibid.). Physical disinfection decreases water pathogens to a certain level depending on the aggressiveness of the treatment. Generally, the target of heat and UV disinfection is the reduction of the initial microorganisms population by 90–99.9% (ibid.). The filtration technique most used is slow filtration because of its reliability and its low cost. The substrates of filtration generally used are sand, rockwool or pozzolana (ibid.). Filtration efficiency is essentially dependent on pore size and flow. To be effective as disinfection treatment, the filtration needs to be achieved with a pore size less than 10  $\mu$ m and a flow rate of 100 l/m<sup>2</sup>/h, even if less binding parameters show satisfactory performances (ibid.). Slow filtration does not eliminate all of the pathogens; more than 90% of the total aerobic bacteria remain in the effluent (ibid.). Nevertheless, it allows a suppression of plant debris, algae, small particles and some soil-borne diseases such as *Pythium* and *Phytophthora* (the efficiency is genus dependent). Slow filters do not act only by physical action but also show a microbial suppressive activity, thanks to antagonistic microorganisms, as discussed in Sect. 14.2.3 (Hong and Moorman 2005; Postma et al. 2008; Van Os 2009; Vallance et al. 2010). Heat treatment is very effective against plant pathogens. However it requires temperatures reaching 95 ° C during at least 10 seconds to suppress all kind of pathogens, viruses included. This practice consumes a lot of energy and imposes water cooling (heat exchanger and transitional tank) before reinjection of the treated water back into the irrigation loop. In addition, it has the disadvantage of killing all microorganisms including the beneficial ones (Hong and Moorman 2005; Postma et al. 2008; Van Os 2009). The last technique and probably the most applied is UV disinfection. 20.8% of EU Aquaponics Hub practitioners use it (Villarroel et al. 2016). UV radiation has a wavelength of 200 to 280 nm. It has a detrimental effect on microorganisms by direct damage of the DNA. Depending on the pathogen and the water turbulence, the energy dose varies between 100 and 250 mJ/cm<sup>2</sup> to be effective (Postma et al. 2008; Van Os 2009).

Physical water treatments eliminate the most of the pathogens from the incoming water but they cannot eradicate the disease when it is already present in the system. Physical water treatment does not cover all the water (especially the standing water zone near the roots), nor the infected plant tissue. For example, UV treatments often fail to suppress *Pythium* root rot (Sutton et al. 2006). However, if physical water treatment allows a reduction of plant pathogens, theoretically, they also have an effect on nonpathogenic microorganisms potentially acting on disease suppression. In reality, heat and UV treatments create a microbiological vacuum, whereas slow filtration produces a shift in effluent microbiota composition resulting in a higher disease suppression capacity (Postma et al. 2008; Vallance et al. 2010). Despite the fact that UV and heat treatment in hydroponics eliminate more than 90% of microorganisms in the recirculating water, no diminution of the disease suppressiveness was observed. This was probably due to a too low quantity of water treated and a re-contamination of the water after contact with the irrigation system, roots and plant media (ibid.).

Aquaponic water treatment by means of chemicals is limited in continuous application. Ozonation is a technique used in recirculated aquaculture and in hydroponics. Ozone treatment has the advantage to eliminate all pathogens including viruses in certain conditions and to be rapidly decomposed to oxygen (Hong and Moorman 2005; Van Os 2009; Timmons and Ebeling 2010; Gonçalves and Gagnon 2011). However it has several disadvantages. Introducing ozone in raw water can produce by-products oxidants and significant amount of residual oxidants (e.g. brominated compound and haloxy anions that are toxic for fish) that need to be removed, by UV radiation, for example, prior to return to the fish part (reviewed by Gonçalves and Gagnon 2011). Furthermore, ozone treatment is expensive, is irritant for mucous membranes in case of human exposure, needs contact periods of 1 to 30 minutes at a concentration range of 0.1-2.0 mg/L, needs a temporal sump to reduce completely from O<sub>3</sub> to O<sub>2</sub> and can oxidize elements present in the nutrient solution, such as iron chelates, and thus makes them unavailable for plants (Hong and Moorman 2005; Van Os 2009; Timmons and Ebeling 2010; Gonçalves and Gagnon 2011).

#### 14.3.2 Biological Methods of Protection

In hydroponics, numerous scientific papers review the use of antagonistic microorganisms (i.e. able to inhibit other organisms) to control plant pathogens but until now no research has been carried out for their use in aquaponics. The mode of action of these antagonistic microorganisms is according to Campbell (1989)), Whipps (2001) and Narayanasamy (2013) grouped in:

- 1. Competition for nutrients and niches
- 2. Parasitism
- 3. Antibiosis
- 4. Induction of diseases resistance in plants

The experiments introducing microorganisms in aquaponic systems have been focused on the increase of nitrification by addition of nitrifying bacteria (Zou et al. 2016) or the use of plant growth promoters (PGPR) such as *Azospirillum brasilense* and *Bacillus* spp. to increase plant performance (Mangmang et al. 2014; Mangmang et al. 2015a; Mangmang et al. 2015b; Mangmang et al. 2015c; da Silva Cerozi and Fitzsimmons 2016; Bartelme et al. 2018). There is now an urgent need to work on biocontrol agents (BCA) against plant pathogens in aquaponics with regard to the restricted use of synthetic curative treatments, the high value of the culture and the increase of aquaponic systems in the world. BCA are defined, in this context, as viruses, bacteria and fungi exerting antagonistic effects on plant pathogens (Campbell 1989; Narayanasamy 2013).

Generally, the introduction of a BCA is considered to be easier in soilless systems. In fact, the hydroponic root environment is more accessible than in soil and the microbiota of the substrate is also unbalanced due to a biological vacuum. Furthermore, environmental conditions of the greenhouse can be manipulated to achieve BCA growth needs. Theoretically all these characteristics allow a better introduction, establishment and interaction of the BCA with plants in hydroponics than in soil (Paulitz and Bélanger 2001; Postma et al. 2009; Vallance et al. 2010). However, in practice, the effectiveness of BCA inoculation to control root pathogens can be highly variable in soilless systems (Postma et al. 2008; Vallance et al. 2010; Montagne et al. 2017). One explanation for this is that BCA selection is based on in vitro tests which are not representing real conditions and subsequently a weak adaptation of these microorganisms to the aquatic environment used in hydroponics or aquaponics (Postma et al. 2008; Vallance et al. 2010). To control plant pathogens and more especially those responsible for root rots, a selection and identification of microorganisms involved in aquatic systems which show suppressive activity against plant pathogens is needed. In soilless culture, several antagonistic microorganisms can be picked due to their biological cycle being similar to root pathogens or their ability to grow in aqueous conditions. Such is the case of nonpathogenic

Pythium and Fusarium species and bacteria, where Pseudomonas, Bacillus and Lysobacter are the genera most represented in the literature (Paulitz and Bélanger 2001; Khan et al. 2003; Chatterton et al. 2004; Folman et al. 2004; Sutton et al. 2006; Liu et al. 2007; Postma et al. 2008; Postma et al. 2009; Vallance et al. 2010; Sopher and Sutton 2011; Hultberg et al. 2011; Lee and Lee 2015; Martin and Loper 1999; Moruzzi et al. 2017; Thongkamngam and Jaenaksorn 2017). The direct addition of some microbial metabolites such as biosurfactants has also been studied (Stanghellini and Miller 1997; Nielsen et al. 2006; Nielsen et al. 2006). Although some microorganisms are efficient at controlling root pathogens, there are other problems that need to be overcome in order to produce a biopesticide. The main challenges are to determine the means of inoculation, the inoculum density, the product formulation (Montagne et al. 2017), the method for the production of sufficient quantity at low cost and the storage of the formulated product. Ecotoxicological studies on fish and living beneficial microorganisms in the system are also an important point. Another possibility that could be exploited is the use of a complex of antagonistic agents, as observed in suppressive soil techniques (Spadaro and Gullino 2005; Vallance et al. 2010). In fact, microorganisms can work in synergy or with complementary modes of action (ibid.). The addition of amendments could also enhance the BCA potential by acting as prebiotics (see Sect. 14.4).

# 14.4 The Role of Organic Matter in Biocontrol Activity in Aquaponic Systems

In Sect. 14.2.3, the suppressiveness of aquaponic systems was suggested. As stated before, the main hypothesis is related to the water recirculation as it is for hydroponic systems. However, a second hypothesis exists and this is linked to the presence of organic matter in the system. Organic matter that could drive a more balanced microbial ecosystem including antagonistic agents which is less suitable for plant pathogens (Rakocy 2012).

In aquaponics, organic matter comes from water supply, uneaten feeds, fish faeces, organic plant substrate, microbial activity, root exudates and plant residues (Waechter-Kristensen et al. 1997; Naylor et al. 1999; Waechter-Kristensen et al. 1999). In such a system, heterotrophic bacteria are organisms able to use organic matter as a carbon and energy source, generally in the form of carbohydrates, amino acids, peptides or lipids (Sharrer et al. 2005; Willey et al. 2008; Whipps 2001). In recirculated aquaculture (RAS), they are mainly localised in the biofilter and consume organic particles trapped in it (Leonard et al. 2000; Leonard et al. 2002). However, another source of organic carbon for heterotrophic bacteria is humic substances present as dissolved organic matter and responsible for the yellow-brownish coloration of the water (Takeda and Kiyono 1990 cited by Leonard et al. 2002; Hirayama et al. 1988). In the soil as well as in hydroponics,

humic acids are known to stimulate plant growth and sustain the plant under abiotic stress conditions (Bohme 1999; du Jardin 2015). Proteins in the water can be used by plants as an alternative nitrogen source thus enhancing their growth and pathogen resistance (Adamczyk et al. 2010). In the recirculated water, the abundance of free-living heterotrophic bacteria is correlated with the amount of biologically available organic carbon and carbon-nitrogen ratio (C/N) (Leonard et al. 2000; Leonard et al. 2002; Michaud et al. 2006; Attramadal et al. 2012). In the biofilter, an increase in the C/N ratio increases the abundance of heterotrophic bacteria at the expense of the number of autotrophic bacteria responsible for the nitrification process (Michaud et al. 2006; Michaud et al. 2014). As implied, heterotrophic microorganisms can have a negative impact on the system because they compete with autotrophic bacteria (e.g. nitrifying bacteria) for space and oxygen. Some of them are plant or fish pathogens, or responsible for off-flavour in fish (Chang-Ho 1970; Funck-Jensen and Hockenhull 1983; Jones et al. 1991; Leonard et al. 2002; Nogueira et al. 2002; Michaud et al. 2006; Mukerji 2006; Whipps 2001; Rurangwa and Verdegem 2015). However, heterotrophic microorganisms involved in the system can also be positive (Whipps 2001; Mukerji 2006). Several studies using organic fertilizers or organic soilless media, in hydroponics, have shown interesting effects where the resident microbiota were able to control plant diseases (Montagne et al. 2015). All organic substrates have their own physico-chemical properties. Consequently, the characteristics of the media will influence microbial richness and functions. The choice of a specific plant media could therefore influence the microbial development so as to have a suppressive effect on pathogens (Montagne et al. 2015; Grunert et al. 2016; Montagne et al. 2017). Another possibility of pathogen suppression related to organic carbon is the use of organic amendments in hydroponics (Maher et al. 2008; Vallance et al. 2010). By adding composts in soilless media like it is common use in soil, suppressive effects are expected (Maher et al. 2008). Enhancing or maintaining a specific microorganism such as *Pseudomonas* population by adding some formulated carbon sources (e.g. nitrapyrin-based product) as reported by Pagliaccia et al. (2007) and Pagliaccia et al. (2008) is another possibility. The emergence of organic soilless culture also highlights the involvement of beneficial microorganisms against plant pathogens supported by the use of organic fertilizers. Fujiwara et al. (2013), Chinta et al. (2014), and Chinta et al. (2015) reported that fertilization with corn steep liquor helps to control Fusarium oxysporum f.sp. lactucae and Botrytis cinerea on lettuces and Fusarium oxysporum f.sp. radicis-lycopersici on tomato plants. And even if hardly advised for aquaponic use, 1 g/L of fish-based soluble fertilizer (Shinohara et al. 2011) suppresses bacterial wilt on tomato caused by Ralstonia solanacearum in hydroponics (Fujiwara et al. 2012).

Finally, though information about the impact of organic matter on plant protection in aquaponics is scarce, the various elements mentioned above show their potential capacity to promote a system-specific and plant pathogen-suppressive microbiota.

## 14.5 Conclusions and Future Considerations

This chapter aimed to give a first report of plant pathogens occurring in aquaponics, reviewing actual methods and future possibilities to control them. Each strategy has advantages and disadvantages and must be thoroughly designed to fit each case. However, at this time, curative methods in coupled aquaponic systems are still limited and new perspectives of control must be found. Fortunately, suppressiveness in terms of aquaponic systems could be considered, as already observed in hydroponics (e.g. in plant media, water, and slow filters). In addition, the presence of organic matter in the system is an encouraging factor when compared to soilless culture systems making use of organic fertilisers, organic plant media or organic amendments.

For the future, it seems important to investigate this suppressive action followed by identification and characterization of the responsible microbes or microbe consortia. Based on the results, several strategies could be envisaged to enhance the capacity of plants to resist pathogens. The *first* is biological control by conservation, which means favouring beneficial microorganisms by manipulating and managing water composition (e.g. C/N ratio, nutrients and gases) and parameters (e.g. pH and temperature). But identification of these influencing factors needs to be realized first. This management of autotrophic and heterotrophic bacteria is also of key importance to sustain good nitrification and keep healthy fish. The second strategy is augmentative biological control by additional release of beneficial microorganisms already present in the system in large numbers (inundative method) or in small numbers but repeated in time (inoculation method). But prior identification and multiplication of an aquaponic BCA should be achieved. The third strategy is importation, i.e. introducing a new microorganism normally not present into the system. In this case, selection of a microorganism adapted and safe for aquaponic environment is needed. For the two last strategies, the site of inoculation in the system must be considered depending on the aim desired. Sites where microbial activity could be enhanced are the recirculated water, the rhizosphere (plant media included), the biofilter (such as in slow sand filters where BCA addition is already tested) and the phyllosphere (i.e. aerial plant part). Whatever the strategy, the ultimate goal should be to lead the microbial communities to provide a stable, ecologically balanced microbial environment allowing good production of both plant and fish.

To conclude, following the requirements of integrated plant pest management (IPM) is a necessity to correctly manage the system and avoid development and spread of plant diseases (Bittsanszky et al. 2015; Nemethy et al. 2016). The principle of IPM is to apply chemical pesticides or other agents as a last resort when economic injury level is reached. Consequently, control of pathogens will need to be firstly based on physical and biological methods (described above), their combination and an efficient detection and monitoring of the disease (European Parliament 2009).

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