Light-Phyllosphere Interactions in Greenhouse Grown Ornamentals

Samareh Gharaie
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Biosystems and Technology
Alnarp

Doctoral Thesis
Swedish University of Agricultural Sciences
Alnarp 2017

Acta Universitatis agriculturae Sueciae
2017:19

Cover: An artistic impression of light (LED)-phyllosphere microbiota
(Sketch: Samareh Gharaie)

ISSN 1652-6880
ISBN (print version) 978-91-576-8811-8
ISBN (electronic version) 978-91-576-8812-5

Print: SLU Service/Repro, Alnarp 2017

Light-Phyllosphere Interactions in Greenhouse Grown Ornamentals

Abstract

Light-emitting diodes (LEDs) have emerged as a promising artificial lighting source in greenhouse production of horticultural crops, as they reduce energy consumption. However, changes in lighting technology are known to affect abiotic and biotic interactions in the phyllosphere, *e.g*. LEDs can change the microclimate within the greenhouse and around the crop, and thus the microbial community structure. Information is lacking on interactions between light spectra and microbiota associated with the canopy, the function of non-phototrophic bacteria associated with the phyllosphere and successful administration of microbial biocontrol agents.

This thesis investigated the impact of different light spectra on plant physiological parameters, microbial community structure, utilisation pattern of energy sources and biosurfactant formation by phyllosphere microbiota in greenhouse-grown ornamentals. A standard protocol for extraction of phyllosphere microbiota, impact of plant species and leaf position, and antagonistic activity of resident phyllosphere microbiota against *Botrytis cinerea* was also studied. Use of culture-dependent methods revealed higher numbers of culturable fungi on basal than on apical leaves, but the numbers did not vary with different light treatments. Metagenomics showed that the fungal microbiome was more diverse on apical leaves. Interactions were found between leaf temperature and many dominant bacterial genera. *In vitro* tests revealed that inhibitory effects of some strains identified by 16S rRNA varied with respect to different media. Phenotypic microarray analysis revealed that light treatments had considerable effects on substrate utilisation by two *Pseudomonas* strains and moderate effects on *Streptomyces griseoviridis*, with blue LEDs having most the pronounced impact. Biosurfactant formation by *Pseudomonas* strains was supported by most substrates when incubated in darkness, but blue LED altered the surface activity more profoundly.

Keywords: 16S rRNA, antagonistic activity, blue light receptor protein, light-emitting diodes, metagenomic analysis, microbial community structure, phenotypic microarray, phyllosphere, Omnilog

Author's address: Samareh Gharaie, SLU, Department of Biosystems and Technology, Microbial Horticulture Unit, P.O. Box 103, SE-23053 Alnarp, Sweden.
E-mail: samareh.gharaie@slu.se

Dedication

To my parents and my sister

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Alsanius, B.W., Bergstrand, K.J., Hartmann, R., **Gharaie, S**., Wohanka, W., Dorais, M. and Rosberg, A.K. (2017). Ornamental flowers in new light: Artificial lighting shapes the microbial phyllosphere community structure of greenhouse grown sunflowers (*Helianthus annuus* L.). *Scientia Horticulturae* 216, 234-247.

II **Gharaie, S**., Windstam, S., Khalil, S., Wohanka, W. & Alsanius, B.W. Isolation and characterisation of epiphytic bacteria from the phyllosphere of greenhouse-grown ornamentals (manuscript).

III **Gharaie*, S**., Vaas*, L.A.I., Rosberg, A.K., Windstam, S., Karlsson, M.E., Bergstrand, K.J., Khalil, S., Wohanka, W. & Alsanius B.W*. (2017). Light spectrum modifies the utilisation pattern of energy sources in *Pseudomonas* sp. DR 5-09. *PLOS ONE* (submitted).

IV Alsanius*, B.W., Vaas*, L.A.I., **Gharaie, S***., Karlsson, M.E., Rosberg, A.K., Grudén, M., Wohanka, W., Khalil, S., & Windstam, S. Dining in blue light impairs the appetite of some leaf epiphytes (manuscript).

*Equally contributing authors

The contribution of Samareh Gharaie to the papers included in this thesis was as follows:

I Partly involved in the writing process.

II Planned the experiment with the co-authors. Performed the experimental work and evaluated the data. Wrote the manuscript with the co-authors.

III Planned the experiment with the co-authors. Performed the experimental work. Evaluated the data with co-authors. Wrote the manuscript together with the co-authors.

IV Planned the experiment with co-authors. Performed the experimental work. Partly evaluated the data and was partly involved in the writing process of the manuscript together with the co-authors.

Abbreviations

CFU	Colony-forming units
DNA	Deoxyribonucleic acid
DGGE	Denaturing Gradient Gel Electrophoresis
HPC	Heterotrophic plate counts
KB	King`s B agar
LED	Light-emitting diode
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PM	Phenotype MicroArray
rRNA	Ribosomal ribonucleic acid
SMs	Secondary metabolites
t-RFLP	Terminal restriction fragment length polymorphism
TSA	Tryptic soy agar

1 Introduction

1.1 Phyllosphere

1.1.1 Phyllosphere microbiome

The plant phyllosphere comprises aerial parts of living plants, including leaves, stems, buds, flowers and fruits, which harbour a large number of diverse microorganisms (Knief *et al.*, 2010; Lindow & Brandl, 2003). Leaves are the most dominant part of the aboveground plant (Vorholt, 2012) and so far most research on phyllosphere microbiology has focused on this dominant aerial structure (Lindow & Brandl, 2003). Microbial populations on plant leaves are diverse, *e.g*. archaea, filamentous fungi and yeasts are known to be present on leaves, but bacteria are considered to be the most abundant phyllosphere inhabitants and can colonise leaves with an average of 10^6-10^7 bacterial cells per cm of leaf surface (Lindow & Brandl, 2003).

The phyllosphere is a hostile habitat for microorganisms, as it is an open system and highly influenced by permanently fluctuating abiotic conditions (Vorholt, 2012; Lindow & Brandl, 2003). Changes in environmental factors, along with plant genotype, can influence the microbial community composition in the phyllosphere (Vorholt, 2012). The composition of the phyllosphere microbial population is thus determined by ability to colonise this environment. Detailed information about abiotic and biotic phyllosphere interactions is given in section 2.2.

1.1.2 Phyllosphere analysis

Several methods for assessing phyllosphere microbiota are available, commonly divided into two main approaches, *viz.* culture-dependent and culture-independent methods. Culture-dependent approaches are based on growing microorganisms on semi-selective medium, whereas culture-independent methods rely on DNA-based methods. One type of culture-

dependent method is the viable plate count technique, in which microorganisms can be plated directly on different nutrient agar (Yang *et al.*, 2001) or brought to suspension from natural samples. Furthermore, suspensions containing the extracted microorganisms can be plated on culture media (Jensen *et al.*, 2013). Identification of single colonies, after culturing on solid culture media, can be performed by biochemical or morphological methods (Jensen *et al.*, 2013) or by gene sequencing techniques (Yarza *et al.*, 2014). Likewise, quantification of microorganisms can be performed by enumeration of the colonies on agar plates and calculation of colony-forming units (CFU) per mL or per g of observed species (Madigan *et al.*, 2012).

Another example of a culture-dependent method is the phenotypic microarray (PM) technique (Biolog Inc., Hayward CA, USA), which is a high-throughput system and can be used for overall analysis of cellular phenotypes of pure cultures or communities in an environmental sample (Line *et al.*, 2011; Bochner *et al.*, 2001). In this technique, pre-filled PM plates are generally used for analysis of cellular pathways in 200 different assays of carbon-source metabolism, 400 assays of nitrogen metabolism, 100 assays of phosphorus and sulphur metabolism, 100 assays of biosynthetic pathways, 100 assays of ion effects and osmolality, 100 assays of pH effects and pH control with deaminases and decarboxylases, and 1000 assays of chemical sensitivity. The chemical sensitivity assays comprise 240 different chemicals, each at four different concentrations. To start a PM assay, two components need to be combined. These are a cell suspension and a nutrient/chemical solution needed to create the 1920 unique culture conditions. The assays are based on a universal culture medium containing all micronutrients needed for cell growth (Bochner, 2009).

Different, assays, *e.g.* PM1 and PM2 representing 190 carbon sources, PM3 representing 95 nitrogen sources and PM4 representing 59 phosphorus and 35 sulphur sources, are commercially available as pre-filled 96-well microtitre plates that provide information on metabolic pathways which are present and active in the cell.

The system is based on phenotypic response to utilisation of the organic sources and the utilisation is monitored by a colour change in a tetrazolium blue-based redox dye (colourless implies that the cells are not able to utilise the organic sources, whereas a colour reaction to purple in the well indicates that cells are actively utilising the substrate) (Bochner *et al.*, 2001). Utilisation rate for each well (colour formation in each well) can be used for cellular phenotype comparison (Bochner, 2003).

The output of this technique is colour-coded kinetic graphs of respiratory response and important biological information is obtained from curve

parameters such as lag phase (λ), steepness of slope (μ), maximum curve height (A) and area under the curve (AUC) (see Supplementary Figure 3 in Paper III).

Culture-dependent approaches, though vastly useful for understanding the physiological potential of extracted organisms, do not necessarily provide complete information on the composition of microbial communities (Onstott *et al.*, 1998). One drawback of these approaches is that, although many different culture media have been designed for recovering as many microorganisms as possible, just a small number of microorganisms can be cultured, while the majority of microorganisms are unculturable (Madigan *et al.*, 2012). Another disadvantage of culture-dependent methods is that fast-growing microorganisms compete with slow growers concerning nutritional requirement (Nocker *et al.*, 2007). Moreover, if the investigation of complex communities is underestimated, then erroneous results can be obtained when counting microorganisms with traditional culture-based methods (Besnard *et al.*, 2000). Therefore different studies suggest combining culture-based and culture-independent approaches in order to obtain comprehensive information on the microbial community (Stefani *et al.*, 2015; Shade *et al.*, 2012; Yashiro *et al.*, 2011). Different culture-independent (DNA-based) methods have been developed for investigation of the phyllosphere microbial community. In past years, a number of methods based on direct amplification and analysis of the small subunit ribosomal RNA gene have been used to study the microbial community of the phyllosphere, *e.g.* denaturing/temperature gradient gel electrophoresis (DGGE) (Rigonato *et al.*, 2016; Reisberg *et al.*, 2012; Delmotte *et al.*, 2009; Yang *et al.*, 2001), terminal restriction fragment length polymorphism (t-RFLP) (Ding & Melcher, 2016; Ding *et al.*, 2013; Penuelas *et al.*, 2012; Hunter *et al.*, 2010; Berg *et al.*, 2005) and high throughput sequencing (next-generation sequencing) (Laforest-Lapointe *et al.*, 2016; Lindahl *et al.*, 2013; Rastogi *et al.*, 2013; Redford *et al.*, 2010).

In culture-independent approaches, the application of high-throughput sequencing techniques has revolutionised scientists' view of microbial communities in environmental samples (Vartoukian *et al.*, 2010). High throughput sequencing techniques are designed for rapid and large-scale microbial community analyses. The five most commonly used methods are: 454-pyrosequencing, Illumina/Solexa, SOLiD, the HeliScope Single Molecule Sequencer and Single Molecule Real Time technology (Morey *et al.*, 2013; Siqueira *et al.*, 2012). The main differences between these technologies are the length of sequences and number of sequence reads achieved (Mardis, 2008).

Illumina, the most commonly used platform nowadays, was employed in some of the work presented in this thesis (Paper I), and is therefore discussed

in more detail here. The Illumina technology was first presented in 2006 and, due to advantages concerning its greater cost-effectiveness and ability to generate larger amounts of data, was quickly accepted by scientists (Hodkinson & Grice, 2015; Caporaso *et al.*, 2012). The Illumina sequencing preparation starts with lengths of DNA that have specific adapters on either end being washed over a flow cell filled with specific oligonucleotides that hybridise to the ends of the fragments. To create a cluster of identical fragments, each fragment is then replicated. Reversible dye-terminator nucleotides are washed over the flow cell and given time to attach, the excess nucleotides are washed away, the flow cell is imaged and the terminators are reversed so that the process can repeat and nucleotides can continue to be added in subsequent cycles (Hodkinson & Grice, 2015). The longest reads that Illumina currently produces, on MiSeq, can produce paired-end reads that are 300 bases in length (Hodkinson & Grice, 2015). In addition, Illumina does not give absolute numbers, but relative abundances. Sequences less abundant than 1% are excluded during the calculation process. Compared with Sanger sequencing, next-generation sequencing methods have greatly reduced the cost and time associated with producing larger amounts of sequenced data (Mardis, 2008). Another advantage of next-generation sequencing techniques is that there is no need to contract clone libraries (Siqueira *et al.*, 2012). However, the length of the sequence reads generated by most next-generation sequence methods is shorter than that required for identification of bacterial gene length (Luo *et al.*, 2012), excluding identification of relative abundances on species level.

Furthermore, sequencing can be used for identification of single colonies produced by culture-dependent methods. Comparative analysis of 16S rRNA gene sequences is important for classification of cultured microorganisms and also for classification of known and novel bacterial genera and species, *e.g.* it enables establishment of taxonomic thresholds for classification of cultured microorganisms and of the many environmental sequences (Hakovirta *et al.*, 2016; Yarza *et al.*, 2014; Mole, 2013; Quast *et al.*, 2013).

1.2 Abiotic and biotic phyllosphere interactions

1.2.1 Abiotic interactions

Phyllosphere colonisation is affected by abiotic factors such as temperature, humidity, water, wind speed and electromagnetic radiation (ultraviolet (UV) and visible light) (Vorholt, 2012; Lindow & Brandl, 2003; Kinkel *et al.*, 2000). In this context, direct impacts of temperature on the development of leaf surface microbiota have been reported (Bernard *et al.*, 2013). Previous research has shown that changes in the temperature conditions and relative humidity

under which plants are grown affect not only the phyllosphere microbial population, but also the ability of pathogens to colonise and survive (Bálint *et al.*, 2015; Brandl & Mandrell, 2002). Similarly, fluctuation in water availability is an important parameter that affects the abundance and diversity of microbial populations (Yadav *et al.*, 2005; Morris & Monier, 2003; Morris *et al.*, 2002). Exposure to different forms of ultraviolet light (UVA, UVB and UVC) can have deleterious effects on leaf microorganisms, contributing to cell death. Phyllosphere microorganisms have developed different tolerance mechanisms towards ultraviolet light, such as pigments and DNA repair systems (Sundin *et al.*, 2002; Kim & Sundin, 2000; Sundin & Jacobs, 1999).

Light plays a key role in multiple phyllosphere interactions. A conceptual structure for light-plant-microbe-environment interactions is presented in this thesis (see Figure 1 in Paper I). This structure describes all the different biotic and abiotic interactions between the plant and plant leaf, abiotic factors and the phyllosphere microbiota, which are discussed in more detail in the following sections.

Light- plant interactions

Light is a fundamental factor for plant growth and development (Li & Kubota, 2009; Fukuda *et al.*, 2008). In this context, three important parameters of light for growth are: quality (spectral distribution), quantity (intensity) and duration. Light quality refers to the spectral distribution of the radiation, *i.e.* wavelength reaching the plant surface. The active part of the light spectrum for plants ranges from ultra-violet to infrared, but the main wavelengths that are absorbed by plant photoreceptors are blue (400-500 nm) and red (600-700 nm) (Huché-Thélier *et al.*, 2015). Light quality has a profound effect on plant growth and greatly influences the anatomy, morphology and physiology of the leaves (Johkan *et al.*, 2012; Macedo *et al.*, 2011; Hogewoning *et al.*, 2010). Light quality effects are species- and cultivar/variety-dependent (Schuerger *et al.*, 1997), but specific light quality can be used to improve the nutritional quality of crops. Absorbed wavelengths can affect the metabolic system of the plants, for instance ultraviolet and blue radiation are involved in the production of secondary metabolites or mechanisms of resistance to pathogens (Huché-Thélier *et al.*, 2016).

Many studies have reviewed the importance of light quality and its effect on growth and development and plant responses to light quality (*e.g.* Folta & Childers, 2008; Devlin *et al.*, 2007). Other studies have examined the impact of LED light on plant yield (Massa *et al.*, 2008) and have reviewed application of LEDs in greenhouse cultivation (Mitchell *et al.*, 2012; Bergstrand & Schüssler,

2010). A recent study examined the effects of light quality on phytochemical accumulation in plants produced in controlled conditions (Bian *et al.*, 2015).

Light intensity is the total amount of light delivered to plants and is usually measured in μmol $m^{-2}s^{-1}$, which is the number of photons of light within the photosynthetic wavelength received by an area of one square metre per second. Light intensity affects photosynthesis, a photochemical reaction which occurs within the chloroplast of plant cells, converting atmospheric CO_2 into carbohydrates (Nishio, 2000). Red light is involved in the development of the photosynthetic system, while blue light is needed for chlorophyll synthesis, localisation of chloroplasts and stomatal opening. Both blue and red light are required for photosynthesis.

Another parameter of light is light duration (photoperiod), which is the period of time per day that plants receive illumination. Photoperiod mainly influences flower bud induction and therefore changing the photoperiod can control flowering time in plants (Singh *et al.*, 2015; Nishio, 2000). In this context, plants have been categorised into two main groups: 1) Short-day plants (SDPs) and 2) long-day plants (LDPs). The SDPs flower when the photoperiod is less than their critical night length, whereas in the LDPs flowering occurs when the day length is longer than their critical night length (Zukauskas *et al.*, 2009; Downs & Thomas, 1982). Furthermore, additional far-red light lowers the red:far-red ratio, which promotes flowering (Runkle & Heins, 2001).

Plants are generally immobile and thus as photosynthetic organisms they must adapt to their biotic and abiotic environments. Therefore they possess diverse photoreceptors sensing ultraviolet-B, ultraviolet-A, blue, red and far-red light in order to deal with their environment. Through these photoreceptors, plants can sense the intensity, quality, direction and duration of light (Kong & Okajima, 2016; Whitelam & Halliday, 2008; Fankhauser & Chory, 1997). The main families of photoreceptors identified so far are phytochromes, cryptochromes and phototropins. They are known as major red, far-red and blue light receptors, respectively (Kong & Okajima, 2016; Chen *et al.*, 2004).

All photoreceptors mediate the photomorphogenesis process in the plant. The signalling pathways of photoreceptors are integrated to adjust the photosynthetic status of the plant to ever-changing environmental light (de Carbonnel *et al.*, 2010).

Light is one of the prominent factors for metabolite production by the plant (Carvalho & Folta, 2014). Besides primary metabolites such as carbohydrates and amino acids, plants produce a vast variety of specialist chemical compounds, which are called secondary metabolites (Wink, 2010). Today many of these secondary metabolites are known to be part of the defence

response of plants against microbes (Joshi *et al.*, 2015) or fungal pathogen and insects (Lattanzio *et al.*, 2006). Previous studies have described the effect of light on production of plant secondary metabolites (Ouzounis *et al.*, 2015; Kopsell & Sams, 2013). Other studies have revealed that blue light can increase the total amount of secondary metabolites (carotenoids) (Kopsell & Sams, 2013; Johkan *et al.*, 2010; Ohashi-Kaneko *et al.*, 2007).

Light-phyllosphere microbiota interactions

Through the process of photosynthesis, light affects the leaf microbiota (indirectly). Photosynthesis is a process orchestrated primarily by light, but also other environmental factors, such as humidity and temperature. Products of photosynthesis, such as organic nutrients, are exuded through the cuticle to the leaf surface and represent important sources of readily available nutrients for phyllosphere microorganisms. Depending on the source and distance of artificial light relative to the plant, it can increase the temperature of the phyllosphere, affect photosynthetic activity and thereby influence the structure of the microbiota.

Like plants, microorganisms can sense and respond to environmental stimuli. Light is a major energy source for phototrophic microorganisms. Hence, many chemotrophic bacteria have the ability to sense light. During their evolutionary history, bacteria have developed elegant photosensory protein modules, which can be categorised into six families, *viz*. rhodopsins, phytochromes, photoactive yellow protein (PYP), light oxygen voltage receptor domain (LOV), cryptochrome and blue light-sensing proteins using (BLUF) (van der Horst *et al.*, 2007; van der Horst & Hellingwerf, 2004). Due to the existence of these light receptors across several bacterial taxa, it has been suggested that light has an unexplored regulatory role in the biology of bacteria (van der Horst *et al.*, 2007; Losi, 2004). In addition to effects of light on the plant that indirectly affect the phyllosphere microbiota, light has direct influences on phyllosphere colonisers. For instance, it has been shown that blue light can influence the physiology of *Xanthomonas axonopodis* pv. citri, and its ability to form biofilms (Kraiselburd *et al.*, 2012). Other studies have suggested that the absence of LOV protein reduces the attachment and biofilm formation of *Caulobacter crescentus* (Gomelsky & Hoff, 2011; Purcell *et al.*, 2007) and that white and blue light can inhibit the motility and attachment of the plant pathogen *Pseudomonas syringae* pv tomato DC3000 (Río-Álvarez *et al.*, 2014). Moreover, it has been reported that blue light positively regulates the swarming activity of *P. syringae* (Wu *et al.*, 2013). Several exciting studies have demonstrated a direct effect of light spectrum on lifestyle options taken

by non-phototrophic bacteria (Losi & Gärtner, 2016; Ricci *et al.*, 2015; Kraiselburd *et al.*, 2012; Gomelsky & Hoff, 2011). A recent study reported that sensing light through two types of photoreceptor can downregulate xanthan production and biofilm formation of *Xanthomonas campestris* pv. *Campestris*, which is a non-photosynthetic phytopathogenic bacteria (Bonomi *et al.*, 2016). In microorganisms, various other cellular responses, such as DNA repair and stress response, can also be regulated through light sensing (Ávila-Pérez *et al.*, 2006; Sinha & Häder, 2002).

1.2.2 Biotic interactions

Plant-microbe interactions

Plants do not exist alone, but co-exist with interacting organisms. Several studies have pointed out the effect of general plant traits such as genotype, (Mason *et al.*, 2014; Vorholt, 2012; Hunter *et al.*, 2010; Whipps *et al.*, 2008; Yang *et al.*, 2001; Kinkel *et al.*, 2000), leaf age (Redford & Fierer, 2009), disease resistance, leaf morphology (shape, trichomes and margin crenulations) (Beattie *et al.*, 2002; Thompson *et al.*, 1993) and leaf chemistry (level of leaf wax, soluble carbohydrate, secondary metabolites and water content) (Vorholt, 2012; Lindow & Brandl, 2003) on phyllosphere microbiota. For instance, the leaf cuticle is hydrophobic, thereby increasing the wettability of the leaf surface, allowing solubilisation and diffusion of substrates and making them available to bacteria. In addition, the wettability of leaves is increased by bacteria producing compounds with surface active properties (Schreiber *et al.*, 2005; Bunster *et al.*, 1989), which facilitates movement of bacteria across the leaf surfaces to areas where nutrients are abundantly present. Nutrients are essential components required for development and growth of all microorganisms. The leaf surface is scarce in nutrients and therefore epiphytic microorganisms face a challenge in obtaining essential nutrients. Nutrient availability is an important limiting factor for microbial growth (Bodenhausen *et al.*, 2014) in the phyllosphere and microbe localisation. Microbial communities may face nutrient limitation due to the waxy cuticle of leaves restricting diffusion of nutrients from inside the leaf to the phyllosphere. As there is no homogeneity in distribution of nutrients in the phyllosphere, this results in patchy distribution of microbes. Results from microscopic analysis show that colonisation is more developed in crevices, epidermal cells and near the trichomes in the proximity of stomata, and along veins (Mariano & McCarter, 1993; Davis & Brlansky, 1991). Leaf-associated microbes may also multiply using exogenous nutrients such as honeydew, pollen, microbial debris

or plant sap (Stadler & Müller, 2000; Warren, 1972). Surface leakages from healthy plants containing metabolites such as carbohydrates, amino acids and organic acids contribute to leaf microbial growth (Tukey Jr, 1970). The incidence of such leakages from plants depends on various factors such as plant species, leaf characteristics (wettability, waxiness, age and duration) and intensity of rain or dew (Tukey Jr, 1970).

Microbe-microbe interaction

Phyllosphere-colonising microbiota do not only inhabit leaf surfaces. Moreover, residential microorganisms may be neutral, beneficial or harmful to plant growth (Lindow & Brandl, 2003), and thus they can act as a promoter or an inhibitor of disease development.

Microorganisms interact with each other (parasitism, mutualism or commensalism) (Kemen, 2014), developing collaboration networks and exchanging common goods. The most common interactions between microorganisms are competition and symbiosis, but they are context-dependent and influenced by abiotic and biotic factors (Hussa & Goodrich-Blair, 2013). It has been reported that the residential microbiome affects the fitness of migrating microorganisms, as well as their location in the phyllosphere. Survival also depends very much on the ability of the community members to optimise collaboration (Kemen, 2014).

Differences in resident phyllosphere microbiota between different plant species may be due to differences such as plant physiochemical characteristics, including the water and nutrient content of the leaves, the levels of phenolics present and leaf and mesophyll thickness.

It has been reported that the majority of random microbe-microbe interactions are competitive rather than co-operative (Foster & Bell, 2012). This resembles the idea of microbial market strategies (exchanging goods, substrate degradation, removing unwanted metabolic products, gene exchange) suggested by another study (Werner *et al.*, 2014). Using living microorganisms as biocontrol agents can reduce infections, *e.g.* several studies have reported that resident phyllosphere microbiota such as *Bacillus* species can suppress growth of the plant pathogen *Botrytis cinerea* in tomato (Kefi *et al.*, 2015) and that some *Bacillus* and *Pseudomonas* species can inhibit growth of the plant pathogen *Escherichia coli* O157:H7 in spinach (Lopez-Velasco *et al.*, 2012). Bacterial strains can also act as biocontrol agents of pathogens, through production of secondary metabolites. Inhibition of plant pathogen growth by secondary metabolites has been reported for *Bacillus amyloliquefaciens* FZB42

(Li *et al.*, 2012; Chen *et al.*, 2009) and for other species of *Bacillus* (Romero *et al.*, 2007).

Phyllosphere microbes tend to have a direct positive influence in altering plant surface properties that may be involved in the fixation of nitrogen (Delmotte *et al.*, 2009), control of plant pathogens (Lopez-Velasco *et al.*, 2012; Lindow & Brandl, 2003) or degradation of organic pollutants (Sandhu *et al.*, 2007). However, some phyllosphere microbes have a negative effect on the host, where plant pathogens may result in disease.

Indigenous and pathogenic phyllosphere microbiota have to cope with nutrient paucity, thereby competing with each other for nutrients and space (Vorholt, 2012). A deeper understanding of how the phyllosphere microbial community is shaped and its stability is yet to be achieved (Vorholt, 2012).

1.3 Greenhouse production of ornamentals and sustainability issues

Ornamental plants cultivated in greenhouses are of high commercial importance and widely used for decorative purposes. Sweden has approximately 1 300 000 million m^2 of total greenhouse area, of which 1.3 million m^2 are used for the production of pot plants and cut flowers and the rest for other vegetables (Jordbruksverket, 2015). Swedish greenhouse production primarily focuses on the following pot plants: geranium, petunia, kalanchoë (*Kalanchoe blossfeldiana*), begonia (*Begonia* sp.), Impatiens (*Impatiens* L.) and poinsettia (*Euphorbia pulcherima*). The latter four, which are considered to be among the principal ornamental crops of importance for the Swedish market, were used as model plants in this thesis.

Most of the profits in the horticulture industry currently derive from greenhouse-grown crops. In recent decades, sustainability is becoming more and more important, along with the climate change. There has been constant and consistent research and innovation in producing a more sustainable growing environment, with a notable focus on sustainable production in greenhouses.

Sustainability cover a wide range of terms. According to Gafsi *et al.* (2006), sustainable agriculture is "*the ability of farming systems to continue into the future*", by helping to conserve natural sources and minimising the environmental impact. In this context, sustainable greenhouse production is highly required (Vox *et al.*, 2010; Opdam *et al.*, 2004), as protected cultivation with widespread use of greenhouses can be unfavourable for the environment (Vox *et al.*, 2010), due to carbon dioxide (CO_2) emissions (Carlsson-Kanyama,

1998) and energy demand (electric energy). The high use of energy is through consumption of electricity for operation of technical systems and for artificial lighting. Therefore the need for sustainable protected systems is likely to increase.

To achieve sustainable greenhouse systems, different strategies such as cultivation technique, suitable equipment management and innovative lighting technology can be applied (Vox *et al.*, 2010). All new innovations aim to make greenhouse production sustainable by decreasing the amount of energy used, reducing the effects of production on the environment, *e.g*. the CO_2 emissions, and reducing management costs (Gadtke, 2010). Many steps have been taken towards improving the sustainability in greenhouses, *e.g*. through a change in the lighting strategy. Greenhouse growers in Sweden are looking for strategies through which lighting costs can be reduced, resulting in control of the amount of light being wasted. One possible way to achieve sustainable lighting is by using LED lights as an alternative to conventional lighting, as they emit light at certain wavelengths that are beneficial for plants (Morrow, 2008). However, the LED technology needs to be optimised in terms of output in order to achieve sustainable and economically viable production (Morrow, 2008). One point that should be kept in mind is that LEDs emit less heat than conventional high pressure sodium (HPS) lamps, which consequently decreases the temperature inside the greenhouse. Therefore heating may be required with LED lighting to maintain leaf temperature at the same levels as with HPS lighting.

The advantages of LEDs compared with conventional forms of lighting are explained in detail in the next section.

Artificial lighting in greenhouse production

Artificial light as a primary energy source for plants can have different sources such as metal-halide, fluorescent and luminous lamps, which are generally used for plant cultivation in greenhouses (Lin *et al*., 2013). For many years, when natural light is not enough for plant production, commercial greenhouse production has used supplementary light. For example, supplementary lighting is often used from autumn to spring in greenhouses at more northern latitudes to increase plant growth and to achieve year-round high production and good plant quality (Paradiso *et al*., 2011; Heuvelink *et al*., 2006) .

High pressure sodium lamps are the dominant source of supplementary light in greenhouse horticulture and are energy efficient as supplementary light sources (Ouzounis *et al.*, 2015; Van Ieperen & Trouwborst, 2007). However, HPS lamps have some limiting traits which restrict the possibilities for their

application in future energy-saving concepts (Opdam *et al*., 2005). For example, they operate at high temperature, resulting in infrared heat emissions to their direct environment (Van Ieperen & Trouwborst, 2007). In addition, low levels of blue light and other photosynthetic wavelengths prevent HPS lamps from being efficient light sources (Marcelis *et al*., 2006). Also, in recent years LED are known to have efficiency up to 2.4 µmol/W, compared to HPS having an efficiency of 2.0 µmol/W (Bergstrand *et al*., 2015). Furthermore, HPS do not provide the possibility for spectral manipulation, which is known to enhance plant growth (Brazaityte *et al*., 2006). As a result, LEDs have attracted considerable interest in recent years and have emerged as an alternative to HPS, due to their longer life span, smaller size, low heat emissions (Schubert & Kim, 2005), scope for control of spectral composition and varying light intensity adaptation (Yeh & Chung, 2009). An LED is a solid state device that is integrated into a digital control system, facilitating a narrow light spectrum (Stutte *et al.*, 2009). One of the advantages of these solid-state light sources is that they can allow selection of wavelengths absorbed by plant photoreceptors that lead to more-optional production, while they can also have beneficial morphological effects on plants (Bourget, 2008; Massa et al., 2008; Morrow, 2008). As a source of light, LEDs have been used for more than 20 years and several studies have reported successful growth of plants under LED lighting (Singh *et al*., 2015; Bula *et al.*, 1991). In LEDs, waste heat is circulated separately from light-emitting surfaces through active heat sinks. This is especially important for high intensity LEDs as light sources that can be placed close to plant leaves, with no risk of over-heating or stressing the crops (Bourget, 2008). Hence, LEDs represent a promising technology for greenhouse production of horticulture plants and lighting systems. However, as mentioned previously, changes in lighting strategy in the greenhouse and crop environment can also lead to changes in the biogeography of the crop. Furthermore, the availability of water on the leaf surface can be affected due to the relationship between air temperature and humidity. The use of LEDs consequently causes a change in the microclimate within the greenhouse and around the crop, with a decrease in both air and leaf temperature and fluctuations in relative humidity. It thereby affects the colonisation pattern of microbial community structure on the crop and also in the cropping system.

1.4 Objectives

The main aim of this thesis was to investigate the interactivity between light spectrum and phyllosphere microbiota in greenhouse-grown ornamentals. Specific objectives of this study were:

- To study the effect of new lighting strategies on the microbiota associated with the canopy of greenhouse-grown sunflower exposed to different artificial light regimes (Paper I)
- To identify a suitable buffer and extraction method and to characterise the epiphytic bacteria of the phyllosphere of greenhouse-grown ornamentals (Paper II)
- To investigate the utilisation of sole energy sources exposed to different light spectra by *Pseudomonas* sp. DR 5-09 as a model strain (Paper III)
- To investigate how multiple environmental stresses affect the respiration of different bacterial strains *in vitro* inhabiting mycelial growth of *Botrytis cinerea* (Paper IV)
- To study how multiple environmental stresses affect biosurfactant formation, as a property for colonisers to explore new habitats (Paper IV)

The hypotheses tested in Papers I-IV were as follows:

- A change in lighting technology (using LEDs instead of HPS lamps) shifts the phyllosphere microbial community structure of greenhouse-grown ornamentals (Paper I)
- The microbial community structure in the phyllosphere of ornamentals differs with respect to the source of artificial lighting and combined effect of leaf age and position (Paper I)
- The choice of buffer and extraction method is decisive for the number of viable counts in the phyllosphere (Paper II)
- *In vitro* antagonistic activity depends on the choice of nutrient medium (Paper II)
- Substrate utilisation patterns depend on light spectrum exposure (Papers III and IV)

- Light spectrum affects the phenotype plasticity of epiphytic phyllosphere colonisers (Paper IV)
- Blue light impairs the respiration pattern of the target strains (Paper IV)
- Responses of bacterial strains to nutrient and light conditions are reflected in their capacity to form biosurfactant colonies (Paper IV)

2 Materials and methods

2.1 Plant material and sampling strategy

In Paper I, sunflower (*Helianthus annuus* cv. 'Teddy bear') was used. For sampling canopy was divided into apical (leaf 1-10, >3 cm leaf length) and basal (leaf 11-16) leaves. For each treatment, leaves were collected from four randomly selected plants and used for laboratory analysis.

In Paper II, four ornamental plants species (*Euphorbia pulcherima* (conventionally and organically grown), *Begonia x hiemalis*, *Impatiens* L. and *Kalanchoë blossfeldiana*, all purchased from a local garden centre in Sweden (PLANTAGEN)), were used.

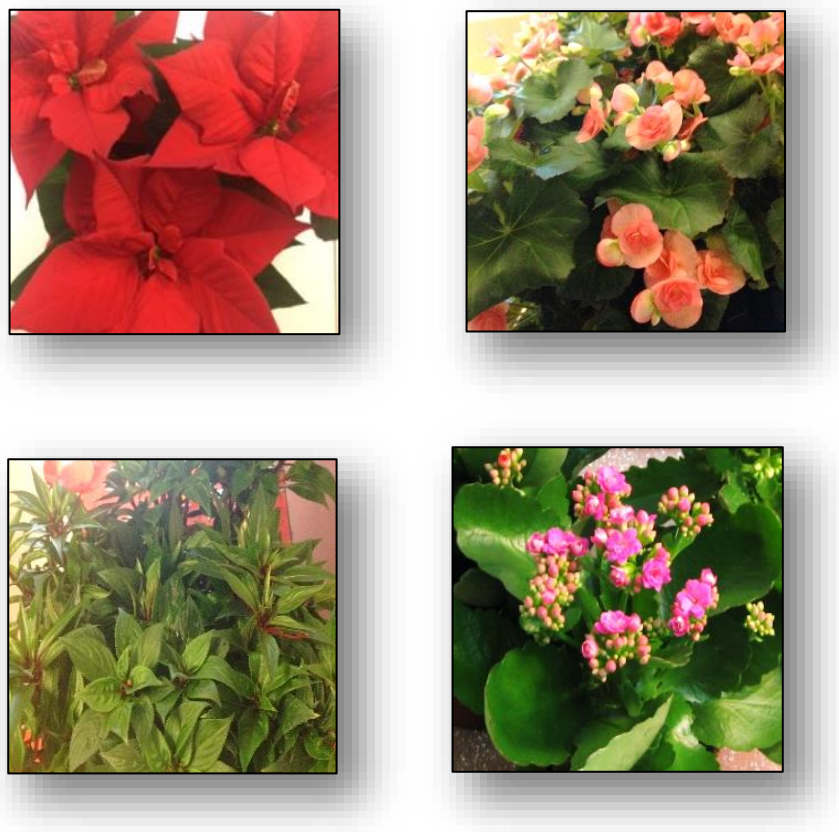

Figure 1. Ornamental plant species used in Paper II: (Top left) *Euphorbia pulcherima*, (top right) *Begonia x hiemalis*, (bottom left) *Impatiens* L. and (bottom right) *Kalanchoë blossfeldiana.* Photo: S. Gharaie

For sampling, the canopy of different species was divided into apical (leaf 1- 5, >2 cm leaf length) and basal (leaf 6-lower) leaves and for each analysis 25 g each of apical and basal leaves were removed.

2.2 Greenhouse experiments

The greenhouse experiment on sunflowers (Paper I) was conducted in a research greenhouse at the Swedish University of Agricultural Sciences, Alnarp, Sweden (55°39N, 013°04E).

For the experiment described in Paper I, sunflower seeds were sown in 35-plug trays (Vefi, Larvik, Norway) filled with peat-based growing medium (K-soil) from Hasselfors Garden AB, Örebro, Sweden and placed in the experimental greenhouse. The seedlings (20 per treatment and experimental unit) were transferred to 13 cm pots after 10 days (same growing medium was used, but amended with 50 g slow-release fertiliser) (NPK 16-6-12, ASB Grünland Helmut Aurenz GmbH, Ludwigsburg, Germany) per 100 L of growing medium. Information about climate settings is provided in Table 1.

Table 1. *Climate settings used in the greenhouse experiments on sunflower (Paper I)*

	Temperature	*Light source*	*Relative humidity*
Greenhouse (experiment 1)	20.3±2.4 °C	White LED red/blue LED High-pressure sodium	62.5±14.6 %
Greenhouse (experiment 2)	18.3±0.7 °C	White LED red/blue LED High-pressure sodium	50.0±7.0 %

2.3 Climate chamber and light treatments (Paper I, III and IV)

For the growth chamber experiments in Paper I, three light treatments were used: 1) white LED (W-LED; 4x90 W, Broham Invest AB, Norsjö, Sweden), 2) red/blue LED (RB-LED; 660 nm, 460 nm; 80:20; 350 W, LightGrow AB,

Helsingborg, Sweden), and 3) high pressure sodium lamps (HPS 400 W, Philips, Eindhoven, The Netherlands).

The photosynthetic photon flux density (PPFD) at canopy level was adjusted to 70-120 μmol m^{-2} s^{-1} by adjusting the distance between the light source and the top of the canopy. Artificial light was given for total of 16 h per day, followed by exposure to natural daylight for 8 h per day.

To check the light transmission through different covering materials in Paper III, the lids of 96-well PM microtitre plates were compared with six other covering materials: Breath-easy sealing membrane, Titer-top sealing film for microplates, sealing tape, household plastic film 1, household plastic film 2 and Greiner viewSeal for 96 plates) (Supplementary Table S1 in Paper I). For this purpose, lid materials were exposed to three different LED sources: (i) white LED, (ii) red LED (660 nm) and (iii) a combination (80/20) of red (660 nm) and blue (460 nm) light.

For the experiments conducted in Paper III and IV, after sealing with the selected covering material (Greiner ViewSeal) PM plates were exposed to three different LED light treatments imposed using blue LED (460 nm), red LED (660 nm) and white LED (covering a continuous spectrum from 350 to 990 nm) (90 W, Trädgårdsteknik AB, Ängelholm, Sweden) (Figure 2).

Figure 2. Cabinet equipped with red, white and blue LED lamps used in experiments in Papers III and IV. Photo: S. Gharaie

2.4 Microorganisms

Microbial strains and their culture conditions

Microbial strains used in Papers II-IV are listed in Table 3. These microbial strains (including three epiphytic bacteria, *Pseudomonas agarici*, *Pseudomonas* sp. DR5-09 and *Bacillus thuringiensis*) were selected from 913 strains of greenhouse-grown ornamentals due to their properties (protease and chitinase activity and biosurfactant formation).

Table 3. *List of microbial trains used in Paper I-IVs and their culture conditions*

Organism	*Medium*	*Incubation time (h)*	*Temperature (0C)*	*Paper*
Pseudomonas agarici	Full strength Tryptic Soy Agar	18	30	III, IV
		72	25	II
Pseudomonas sp. DR5-09	Full strength Tryptic Soy Agar	18	30	III, IV
		72	25	II
Bacillus thuringiensis	Full strength Tryptic Soy Agar	18	30	III, IV
		72	25	II
**Streptomyces griseoviridis (CBS 904.68)*	Oatmeal Agar	192	25	IV
**Botrytis cinerea (CBS 120092)*	Potato Dextrose Agar	192	25	II, IV

* Purchased from Centraalbuureau voor Schimmelcultures, Utrecht, The Netherlands

2.5 Analyses

2.5.1 Plant analysis (Paper I)

Measurements of plant height, width, number of nodes and shoot length (non-destructive measurements), and fresh and dry plant weight (destructive measurements) in Paper I were performed at the end of the experiment on 10 plants per treatment. Dry weight was measured after two days of desiccation at 75 °C.

To have a consistent daily light integral for each treatment and experiment in Paper I, light intensity was measured at the canopy level using a light meter (Delta HD 2302.0, probe LP 471, Delta OHM, Padua, Italy) and light quality was observed with a spectrophotometer (Delta HD 2302.0, probe LP 471, Delta OHM, Padua, Italy). For measuring the rate of photosynthesis and chlorophyll fluorescence, the third fully developed leaf from the apex was used. The rate of photosynthesis was measured using a leaf chamber photosynthesis meter (LC Pro+, ADC BioScientific Ltd, Hoddesdon, UK) and chlorophyll fluorescence using a PAM-2500 fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Measurements of leaf temperature (top leaf) were performed using an infrared camera (Flir IX, Flir systems Inc., Wilsonville OR, USA).

2.5.2 Extraction of microbiota from the phyllosphere (Paper I and II)

For Papers I and II, apical and basal leaves were randomly removed from selected plants. Further leaf samples were washed as described in Paper I and II and used for plate counts, characterisation of enzyme activity and production of biosurfactant, dual culture test, phenotypic microarray, sequencing and meta-genomic analysis.

In order to store the microbial strains before testing (*e.g*. characterisation of enzyme activity and production of biosurfactant, dual culture test, phenotypic microarray and sequencing) in Paper II, strains were transferred to cryo medium (described in detail in Paper II) and stored at -80 °C. In Paper I, for metagenomics analysis the wash solution from leaf samples was directly transferred to a sterile tube and after centrifugation and resuspension preserved at -80 °C.

2.5.3 Culture-dependent microbial analyses

Viable count

Aliquots from the leaf wash-off were serially diluted and plated on various growing media in order to assess a wide range of phyllosphere microorganisms (Table 2). Total viable bacteria and fungi were counted as colony-forming units (CFU) per mL and per g fresh weight (fw) (Papers I and II).

Table 2. . *Overview of plate count analysis (semi-selective media and incubation conditions) for enumeration of phyllosphere microorganisms (Papers 1 and II)*

	Diluted Tryptic Soy Agar (0.1x TSA)	*Standard I nutrient agar (SN1)*	*Standard II nutrient agar (SN2)*	*King B agar (KB)**	*Diluted Malt Extract Agar (0.5x MA)*
Plate counts	Total culturable bacteria	Total culturable bacteria	Total culturable bacteria	Fluorescent pseudomonas	Total fungi
Incubation time (h)	72	72	72	48	96
Incubation temperature(^{0}C)	25	25	25	25	25
Product number	Difco 218263	Merck 105450	Merck 107883	-	Difco 218630

* King *et al*., 1954.

Screening for enzyme activity (Paper II)

Isolated strains from the phyllosphere of greenhouse-grown ornamentals after pure culturing and storing were screened for their enzyme activity (protease and chitinase).

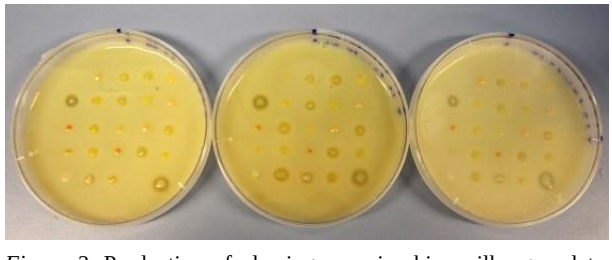

Figure 3. Production of clearing zone in skim milk agar plates by isolated strains. Photo: S. Gharaie

For determination of protease (Figure 3) isolated strains were inoculated on skim milk agar (SMA) (Smibert *et al*., 1994), while for chitinase activity they were plotted on colloidal chitin minimal agar (CCMA) (Renwick *et al*., 1991), with 24 strains spotted on each agar according to a prepared template. The 25th position was used as a control. Plates were sealed, and halo formation assessed after 24, 72 and 96 h at 30 °C.

Screening for biosurfactant formation (Papers II and IV)

For detection of biosurfactant production in Paper I, isolated strains after pure culturing were assessed by drop-collapse test (flat drops indicated positive for biosurfactant formation, whilst cultures with convex drops were recorded as negative for biosurfactant formation) (Youssef *et al.*, 2004).

In Paper IV, PM panels after inoculation with selected microorganisms (*Pseudomonas agarici*, *Pseudomonas* sp. *DR5-09, Bacillus thuringiensis and Streptomyces griseoviridis*) and exposure to different types of LED light were checked for detection of biosurfactant formation.

Dual culture test (Paper II)

Isolated strains that tested positive for chitinase activity and biosurfactant formation were selected for dual culture test to evaluate their antagonistic activity against *Botrytis cinerea* (CBS 1290092, Utrecht, Netherlands). Candidate strains were inoculated on potato dextrose agar (PDA), King B agar (KB) and 0.1x tryptic soy agar (TSA) (all Difco, Michigan, USA) on either side of petri dishes (90 mm petri dish, 10 mm distance from the edge of the petri dish) two days before inoculation of *B. cinerea* and thereafter the pathogen (5 mm plug) was inoculated in the centre of the petri dishes. The plates were incubated at 25 °C. The inhibition zone was measured after mycelium reached the edge of the agar plates. Inhibition (%) was determined according to an existing method (Skidmore & Dickinson, 1976).

Phenotypic microarray

In order to investigate the impact of light spectrum on utilisation of different sole energy sources by phyllosphere microbiota, in Papers III and IV microbial utilisation of energy sources was examined using Phenotype MicroArray (PM) panels PM01, PM02, PM03 and PM04 (Biolog Inc., USA). The PM panels are commercially available pre-filled 96-well microtitre plates

containing 190 different carbon sources (PM01 and PM02), 95 different nitrogen sources (PM03), and 59 phosphorus and 35 sulphur sources (PM04). The PM method was performed according to the manufacturer's recommended protocol. PM01 and PM02 were incubated as sole substrate, whereas PM03 and PM04 were supplemented with 2 mM sodium succinate and 2 μM ferric citrate as additional carbon sources (enrichment). Microorganisms used for PM assay and their culture conditions are described in detail in section 3.4. After inoculation, the PM plates were sealed with selected covering material (Greiner ViewSeal) and incubated in the Omnilog reader (Omnilog, Biolog Inc., USA) in dark conditions or exposed to different light spectra (blue, red or white LED). Detailed information regarding incubation time, temperature, experimental set-up and data collection can be found in Papers III and IV.

2.5.4 Culture-independent analyses

Sequencing

The isolates displaying antagonistic activity were identified using 16S rRNA gene sequencing in Paper II. The cryo-preserved cultures were grown on full-strength TSA, incubated overnight at 30 °C and thereafter processed for DNA extraction as described in Paper II. The PCR analysis of the 16S rRNA genes was performed using the universal forward primer ENV1 and the reverse primer ENV2. Amplicons with the correct size of amplified fragments (1500 bp) were sent to Eurofins MWG (Ebersberg, Germany). The primer used by Eurofins MWG was ENV1.

Metagenomic analyses

Bacterial and fungal communities of sunflower leaves were investigated using Illumina. For Illumina analysis, the wash solution was processed as described in Paper I. The pellets obtained were used for the extraction of genomic DNA (King Fisher Cell and Tissue DNA Kit, Product number: 97030196, Thermo Fisher Scientific Oy, Vantaa, Finland). The DNA construction of amplicons of interest was determined by gel electrophoresis. The amplicon pools were purified to remove primer, and additional purification on MinElute columns (Qiagen) was also performed. Purified amplicon pool DNA was used for constructing Illumina libraries. Illumina data were analysed by the bioinformatics service of LGC Genomics, Berlin, Germany, using QIIME1.8.0.

2.6 Calculations and statistics

General linear model (GLM) analysis, followed by Tukey's test ($p<0.05$), stepwise regression and cluster analysis (single linkage method), were performed with Minitab (State College, PA, USA, Version 16.2.4) software and biodiversity indices (Shannon H, Chao1) and Euclidian distance were computed using the paleontological statistics software PAST (version 3) in Paper I. Analysis of variance (ANOVA) in Papers II-IV was carried out using Mintab (State College, PA, USA, Version 16.2.4) software. Phylogenetic comparison in Paper II was conducted using Ribosomal Database Project, release 11 (RDP, Michigan State University, East Lansing, USA). The PM data in Papers III and IV were recorded using the OmniLog® PM kinetic analysis software (Product Number UA24331-PMM, version 1.6), and thereafter analysed using the R statistical software (Team, 2016) and functionality from the dedicated R package opm (Vaas *et al.*, 2013). Calculation of principal component analysis (PCA) in Paper IV was performed using Minitab vers.17 (Minitab Inc., State College Pennsylvania). Detailed information on the statistical methods used is provided in each individual Paper (I-IV).

3 Results and discussion

3.1 Microbial community structure in greenhouse-grown ornamentals (Papers I and II)

The phyllosphere is an ecologically and economically important ecosystem that hosts a variety of microbial communities. Phyllosphere microbiota play a critical role in protecting plants from diseases, as well as promoting their growth by various mechanisms. There are gaps in our understanding of how and why microbiota composition varies across spatial and temporal scales. There is also a lack of knowledge regarding the ecology of leaf surface colonisers, their interactions with their hosts and the genetic adaptations that enable phyllosphere survival of microorganisms.

3.1.1 Effect of light spectrum on phyllosphere microbiota

In this thesis (Paper I), the effect of light spectrum on microbial communities associated with the leaf microbiota of ornamental sunflower (*Helianthus annuus*) grown in the greenhouse was examined.

The viable count results showed that light treatment had no effect on viable counts of bacteria and fungi (Figure 5B in Paper I). However, there were significant differences in viable counts between the different leaf positions on all semi-selective media (Figure 5A in Paper I).

Leaves can be colonised by 10^3-10^6 culturable fungi and 10^6-10^9 bacteria (Timms-Wilson *et al.,* 2006). However, in Paper I the size of the bacterial epiphytic populations was smaller, while the fungal counts were within the reported range. The low viable counts of bacteria observed in Paper I might be due to the extraction method used. Similar bacterial epiphytic population size was found in Paper II using the same extraction method. Although viable counts gave interesting information, it should be considered that culture-

dependent methods (viable counts) are inadequate to reflect the entire phyllosphere microflora (Whipps *et al.*, 2008; Yang *et al.*, 2001). Therefore, for investigation of microbial community composition exposed to different light treatments in more detail, Illumina was used as a culture-independent method, as a complement to the culture-dependent method.

Metasequencing of the fungal community indicated that different light treatments affected species abundance and evenness, but not species richness (Chao1) (Table 2 in Paper I). Irrespective of the light treatments, *Ascomycota* was the dominant fungal phylum (Figure 6 in Paper I). On phylum level, significant differences were observed between the two LED light treatments ($p=0.028$; $N=15$) for *Ascomycota*. Its share within the fungal microbiome of sunflower leaves was highest when exposed to white LEDs (98.1%) and lowest when exposed to red-blue LED light (93.5%) (Figure 6B in Paper I). No significant differences were observed for the relative abundance of *Ascomycota* on sunflower leaves between HPS and LED treatments (red-blue and white LED) (Figure 6A in Paper I). However, in the case of *Basidiomycota*, significant differences were seen for the leaves exposed to white LED light for relative abundance, and differences were seen between the two LED treatments ($p=0.036$). There were, however, no differences in the case of *Zygomycota* or miscellaneous phyla for either light treatment or leaf position. No interactions between light treatment and leaf position were found for any of the phyla. Distribution of fungal classes in the phyllosphere of sunflower was affected by different light treatments (Figure 6A-C in Paper I) and leaf position (Figure 6D-E in Paper I).

The dominant class in the fungal microbiome of the sunflower phyllosphere was *Dothideomycetes* when treated with HPS lamps. Its relative abundance was decreased when exposed to LEDs. The share of both *Leotimycetes* and *Sordariomycetes* was higher when exposed to LEDs (Figure 6A-C in Paper I). In general few statistical differences were observed for the impact of light treatment and the leaf position on the fungal microbiome of greenhouse-grown sunflower.

On phylum level, there were no significant differences in bacterial community in the phyllosphere except for the group of non-classified bacteria. With respect to altered light treatment, Gammaproteobacteria (34-37%), Alphaproteobacteria (18-23%), Betaproteobacteria (10-12%), Actinobacteria (8.6-10.6%) and Sphingobacteria (5.2-5.7%) were the most dominant taxa. On order level, no impact of light treatment or leaf position was observed, except for Xanthomonadales. The impact of light treatment on some bacterial genera associated with sunflower leaves was indirect, through the interaction between

leaf temperature, stomatal conductance and chlorophyll fluorescence (Figure 1 in Paper I).

The results from the first experiment performed in Paper I confirmed the impact of light spectrum on the phyllosphere microbiota, which is consistent with previous findings (Itagaki *et al.*, 2016; Schuerger & Brown, 1994). Interestingly, however, we found high colonisation by *Golovinomyces* and *Podosphaera* (causative agents of powdery mildew; Mulpuri *et al.*, 2016; Chen *et al.*, 2008; Braun, 1995) in the sunflower phyllosphere. Colonisation was highest on canopies treated with white LEDs, and considerably lower when the canopies were exposed to HPS and red-blue LEDs. A previous study (Suthaparan *et al.*, 2010) has reported a reduction in conidia germination of *Podosphaera pannosa* on greenhouse roses when exposed to blue LEDs in detached leaf assays, while a combination of red LED with 18 h of white LED treatment followed by 6 h of red LEDs inhibited conidia formation in whole plant tests. These results are in line with those in Paper I and support the initial hypothesis regarding the effect of light spectrum quality on leaf microbiota.

The study reported in Paper I was the first to investigate the interaction between light treatment, plant physiological properties and resident microbiota of greenhouse-grown sunflower. It showed that the effect of light treatment on phyllosphere microbiota (fungi species abundance and evenness) was mostly due to different leaf temperatures under LEDs compared with HPS. Moreover, no direct effects of light treatment were seen on photobiology parameters, but there were correlations between these parameters and important bacterial and fungal genera such as *Bradyrhizobium*, *Sphingomonas*, *Brevibactericum*, *Bacillus*, *Hypotrachyna* and *Aureobasidium*. In addition, the effect of light treatment on fungi was direct, whereas bacteria were affected indirectly through plant environment fluctuations.

3.1.2 Occurrence of bacterial antagonistic to *Botrytis cinerea* (Paper II)

Botrytis cinerea is a necrotrophic fungal pathogen and causal agent of grey mould, which is one of the most widespread fungal diseases, attacking over 200 plant species, including ornamentals. This pathogen causes substantial commercial crop losses every year (Rupp *et al.*, 2016; Hahn, 2014; Dean *et al.*, 2012; Williamson *et al.*, 2007). It also has unlimited adaptability under broad environmental conditions.

One of the aims of this thesis was to develop an optimal extraction methodology to evaluate the phyllosphere microbiota of greenhouse-grown ornamentals (Paper II). The method developed was then used for screening

bacteria with ability for enzyme activity, biosurfactant production and *in vitro* antagonism towards *Botrytis cinerea*.

A suitable extraction procedure was selected through determination of the impact of ultrasonic treatment, different buffers (PPB and TRIS) and microbiological medium (TSA, SN1 and SN2) on the number of culturable bacteria inhabiting the phyllosphere of model plant *Begonia x elator* (see Materials & Methods section in Paper II). As can be seen from the results (Figure 1 in Paper II), choice of buffer had a significant effect, with PPB causing no difference in viable counts on the three nutrient media tested (0.1x TSA, SN1 and SN2). Furthermore, following sonication the number of viable counts significantly declined in comparison with the non-sonicated treatment.

Heterotrophic plate counts (HPC) displayed considerable differences between the model crops within apical and basal leaves (Figure 2 in Paper II). For instance, viable counts were significantly higher for *Impatiens*, in both apical and basal leaves, but not for fluorescent pseudomonads. In the case of HPC, higher counts were observed for basal leaves of conventionally grown Poinsettia compared with its organically grown counterpart.

A total of 913 bacterial strains displaying morphological differences were collected from apical and basal leaves of *Poinsettia* (conventionally and organically grown), *Begonia*, *Impatiens* and *Kalanchoë*. All these bacterial strains were screened for some antagonistic properties (protease and chitinase activity and biosurfactant formation) against *B. cinerea.*

The phyllosphere is a dynamic environment, subjected to variations in environmental factors, nutrient and water availability, plant species differences and leaf age (Vorholt, 2012; Hunter *et al.*, 2010; Redford & Fierer, 2009; Whipps *et al.*, 2008; Lindow & Brandl, 2003; Jager *et al.*, 2001; Kinkel, 1997). Resident microbial communities in the microenvironment provided by the leaves differ considerably, depending on a variety of these factors affecting the phyllosphere. Previous studies have suggested that bacterial colonisation and bacterial distribution in the phyllosphere may also be governed by plant species (Lambais *et al.*, 2014; Vokou *et al.*, 2012; Yadav *et al.*, 2004; Yang *et al.*, 2001). Heterotrophic plate counts (Paper II) for *Impatiens* (apical and basal leaves) and conventionally grown *Poinsettia* (basal leaves) had the highest viable counts amongst the plants tested. It was therefore concluded that significant differences in HPC between apical and basal leaves for different model crops are probably due to species differences. These results, representing the effect of plant species on bacterial counts on apical and basal leaves, are in agreement with findings in previous studies (Knief *et al.*, 2010; Yang *et al.*, 2001).

Most of bacteria (in terms of number) displaying enzyme (protease and chitinase) and biosurfactant activity were retrieved from basal leaves of *Impatiens*. Previous studies have suggested that bacteria displaying enzyme and biosurfactant activity have potential as biocontrol agents (Kefi *et al.*, 2015; D'aes *et al.*, 2010; Hultberg *et al.*, 2010; Trotel-Aziz *et al.*, 2008; Soberón-Chávez *et al.*, 2005). These bacterial strains can degrade fungal cell walls by production of chitinase (Kefi *et al.*, 2015; Kim *et al.*, 2012; Yan *et al.*, 2011) or inhibit fungal growth through formation of biosurfactants (Varnier *et al.*, 2009), which are biologically produced amphiphilic compounds that exhibit surface activity through the actions of their hydrophilic and hydrophobic groups (Burch *et al.*, 2011). As most of the epiphytic colonisers with antagonistic properties identified in this thesis were retrieved from basal leaves of *Impatiens*, an effect of plant species and leaf age was also observed. This indicates that these factors might affect the abundance of bacterial strains with biocontrol properties in the phyllosphere, but more research is necessary to confirm this.

In Paper II, a dual culture test on the three semi-selective media (0.1x TSA, KB and PDA) was employed to check the inhibitory effect of candidate strains displaying chitinase activity and biosurfactant formation against *B. cinerea*. To determine the nutritional effect of three semi-selective media on the inhibition activity, comparisons were made between these media. The results showed that mycelial growth of *B. cinerea* was inhibited by some selected strains among the 67 strains examined. For instance, strain PCb52T extracted from the basal leaves of conventionally grown Poinsettia showed the strongest inhibition against *B. cinerea* on 0.1x TSA, whereas on KB, strain Ia176K, which was isolated from apical leaves of *Impatiens*, displayed the strongest inhibition. Mycelial growth of *B. cinerea* when grown on PDA was also strongly inhibited by strain Ib44K extracted from basal leaves of *Impatiens*. On comparing the different media, it was found that the inhibitory effect was significantly higher on some media for some strains, such as Ib44K on PDA compared with KB (Table 3 in Paper II). A previous study has also reported inhibitory effects on fungi by bacterial biocontrol agents, in a dual culture test conducted on two forms of nutrient media (PDA and 0.1x TSA) (Sylla *et al.*, 2013). This differential influence of various nutrient media on inhibitory effect might indicate an effect of nutrient composition.

The strains with biosurfactant production, chitinase activity and *B. cinerea*-inhibiting properties *in vitro* were identified with 16S rRNA sequencing. Proteobacteria, Actinobacteria and Firmicutes were the three major phyla to which the identified isolates belonged (Table 4 in Paper II). Furthermore, nine isolates with *in vitro* properties to inhibit mycelial growth of *B. cinerea* were

identified (*Pseudomonas vancouverensis*, *Pseudomonas asplenii*, *Pseudomonas segetis*, *Pseudomonas mosselii*, *Pseudomonas reinekei*, *Bacillus subtilis*, *Rhizobium rosettiformans*, *Paenibacillus taichungensis* and *Enterobacter kobei*). Among these nine strains, only *B. subtilis* has previously been reported in this context (Kefi *et al.*, 2015). Although that study also concluded that strains with antagonistic properties might affect the suppression of *B. cinerea* (Kefi *et al.*, 2015), it should be noted that as there were no interactions *in planta* in this thesis and as results were obtained from controlled conditions, the underlying mechanisms need further research.

3.2 Impact of light spectrum on utilisation of energy sources by selected phyllosphere bacteria (Papers III and IV)

There is limited information about the impact of light spectrum on the functionality and composition of non-phototrophic bacterial phyllosphere biota. Therefore, Papers III and IV investigated the utilisation pattern of energy sources in selected phyllosphere microbiota under different LED spectra. An *in vitro* method was developed to study phenotypic profile responses of pure bacterial cultures to different LED regimes by modification and optimisation of a protocol for the Phenotype MicroArray™ technique (Paper III).

Pseudomonas sp. DR 5-09 was used as a representative bacterial strain model in Paper III, as it tested positive for protease, chitinase and biosurfactant properties in screening in Paper II. Paper III examined the utilisation of C, N, P and S sources as a function of maximum curve height under different light regimes. In addition to darkness (considered control conditions), blue (460 nm), red (660 nm) and white (350-990 nm) LEDs were used.

Substrate utilisation assays were conducted using four pre-fabricated panels consisting of 379 substrates and conditions (190 C sources, 95 N, 59 P, 35 S). Responses generated for the 190 C sources provided a basis for distinguishing patterns for each light regime included in the study.

Carbon utilisation in darkness and in red light incubation regime had least influence on the maximum curve height, and these clustered together compared with white and blue light. In addition, blue light incubation for sole C sources deviated from all other light treatments. In general, the blue light spectrum had the most decisive impact on substrate utilisation of C sources.

Similarly, a strong impact of blue light spectrum on respiration was recorded for N utilisation. Different N sources (*e.g.* L-theronine, D-asparagine, L-isoleucine, cytosine, D,L-α-amino-N-butyric acid, D-mannosamine and nitrate) were affected under all light conditions except blue light. Overall,

under blue light N utilisation was significantly lower in terms of maximum curve height of dark conditions.

Similarly, P utilisation pattern showed significant differences for blue light compared with red, white and dark incubation conditions. Of 59 P sources included, 53 sources differed significantly under blue light incubation, while the sources were metabolised to the same extent under all other light regimes.

Further analysis on the effect of selected wavelengths on energy source utilisation by *Pseudomonas* sp. DR 5-09 revealed that reduced substrate utilisation was due to the restrictive effect of light exposure on metabolic pathways. Blue light interferes with several major critical metabolite pathways. With respect to the 379 different substrate and conditions, no general effect of light regime on substrate utilisation was observed. Through KEGG pathway analyses, it might be possible to identify which pathways are affected. It is important to understand the consequences of inhibited pathways on the general utilisation of substrates.

Substrate utilisation by *Pseudomonas* sp. DR 5-09 was studied under different light treatments. Some of the substrate sources were provided as sole substrate and some were enriched with C sources (Figure 6 in Paper III). For some of the sources, such as thymidine and D-aspartic acid, utilisation was not affected irrespective of light regime and nutritional status. However, some other sources, such as L-lysine, D-serine and D-glucose-6-phosphate, showed considerable utilisation when they were provided as enriched compared with sole substrate. Utilisation of substrate was the other way around, with higher utilisation observed for sole substrate compared with enriched. Thus no general pattern was observed for energy source utilisation of substrates when they were provided as enriched sources. In a previous study on *Pseudomonas aeruginosa*, succinate and some other sources, such as L-aspartate, glycerol, L-glutamate, L-asparagine, fumarate, α-ketoglutarate and L-glutamine, were found to be the main and most preferred C sources due to their positions in the citric acid cycle (Li & Lu, 2007). In contrast, the results obtained in this thesis showed higher levels of respiration for C sources such as L-aspartic acid, L-arginine, putrescine, L-pyroglutamic acid, L-serine, L-glutamine, L-asparagine, L-proline and L-glutamic acid, even in the absence of succinate. Hence there is a need for further studies to identify the role of these C sources in different conditions.

It is not possible to draw any conclusions based on nutrient utilisation, since it also depends on other environmental factors. The results from this study showed that utilisation was most likely higher in enriched conditions than sole substrate conditions. This supports previous findings by Li and Lu (2007). Low utilisation of some of the substrates when provided as the sole source in this

thesis might be explained by a lack of uptake mechanisms or absence of essential nutrients. The ATP-binding cassette (ABC) uptake systems are the largest transport systems in bacteria. Almost all of the less-preferred sugars in bacteria are transported by the ABC uptake systems (Rees *et al.*, 2009; Higgins, 1992). A recent study (Maqbool *et al.*, 2015) demonstrated that this substrate-binding protein is substrate-specific and has high affinity for the ABC uptake systems.

Carbon catabolite repression (CCR) is a regulatory process in many bacteria such as the *Pseudomonas* genus (Rojo, 2010). This process assists in fast adaptation to the competitive environment by utilising preferred C sources for energy generation (Görke & Stülke, 2008). It works as a regulatory mechanism that inhibits the synthesis of enzymes for less-preferred sources and overexpression of virulence genes that help the bacteria to access new nutrient sources (Moreno *et al.*, 2009; Görke & Stülke, 2008). Hence, CCR has been shown to be a driving force for evolution in many Gram-negative bacteria, including both free-living and pathogenic strains. In this study succinate was used as a preferred C source, as in other studies (Li & Lu, 2007; Collier *et al.*, 1996). The molecular mechanisms in the genus *Pseudomonas* are not completely understood, but it is most likely that CCR or reverse CCR activates a regulatory mechanism for nutrient uptake.

Nutritional status might also be an important factor for substrate utilisation. Based on previous studies (Beier *et al.*, 2015; Werner *et al.*, 2014) and this results, one could conclude that in addition to light spectrum, nutritional factors play an important role in the microbial phenotypic response.

Paper IV examined light-dependent phenotypic plasticity in phyllosphere bacterial strains in terms of substrate resource utilisation upon exposure to different light regimes. The strains tested were *Pseudomonas agarici*, *Pseudomonas* sp. DR 5-09 (results already discussed above), *Bacillus thuringeinsis* serovar *israeliensis* and *Streptomyces griseoviridis*. The results indicated a distinct impact of light regime on substrate utilisation by *Pseudomonas agarici*, *Pseudomonas* sp. DR 5-09 and *Streptomyces griseoviridis* (Figure 4 in Paper IV) as follows: (i) In the case of *P. agarici* (C sources) and *S. griseoviridis* (C, N, P and S sources), blue LED and white LEDs differed from red LED and dark incubation. (ii) In the case of *P. agarici* (P and S sources), blue and red LED differed from white LED and dark incubation. (iii) For N sources for *P. agarici*, dark incubation had a distinctly different response from all LED regimes. (iv) In the case of *B. thuringiensis* (all sources), no responses to light regime were observed.

Apart from these main findings, differences were seen for the different substrate x light interactions for each specific strain. As previously mentioned,

for *Pseudomonas* sp. DR 5-09 light regime and enrichment conditions significantly affected substrate utilisation. For example, in the case of *P. agarici*, utilisation of substrate, when provided as both sole and enriched, discriminated the impact of blue LED from that of all other light regimes.

No Impacts of light regime were observed for energy source utilisation of sole and enriched substrates by *B. thuringiensis*. Better utilisation was observed for most of the substrates when they were offered as sole C sources. Regarding the fourth strain (*S. griseoviridis*), the impact of light regime on substrate utilisation was less noticeable than for *P. agarici*.

In further analyses, the two Gram-negative strains (*Pseudomonas*) were compared regarding their response as light-dependent phenotypic effects. Differences were mostly found for C sources when they were incubated in dark conditions. For S sources, exposure to red LED and incubation under blue LED decreased the differences in substrate utilisation.

Comparisons between the four selected strains revealed that not all differences between these strains were dependent on light regime. Overall, the results indicated that blue light impairs the utilisation of sole and enriched substrates by the two selected *Pseudomonas* strains and has similar, but less pronounced, implications for *S. griseoviridis*. However, substrate utilisation by *B. thuringiensis* appears to be indifferent to light regime during incubation (Figures 4, 6-7).

Evolutionary responses to environmental changes are mainly influenced by different environmental conditions (Salinas & Munch, 2012; Wong & Ackerly, 2005). Therefore microorganisms, besides having a versatile metabolism which is linked to tight but flexible regulation of the expression of metabolic pathways that directly optimise efficiency and ecological fitness (already discussed), try to use other modes of adaptation to variable environments. Phenotype plasticity is one of these modes of adaptation used by organisms and suggested in many other fields, such as zoology (Furness *et al.*, 2015; Miner *et al.*, 2005). Adaptive phenotypic plasticity is a common phenomenon by which a genotype can create different phenotypes from a single genotype depending upon environment (Ghalambor *et al.*, 2007; Sultan & Stearns, 2005; Bradshaw, 1965). The hypothesis tested in this thesis was that light, as one of the environmental cues, can affect the phenotype plasticity of epiphytic phyllosphere colonisers. A previous study in another field reported that environmental factors such as light and temperature can induce phenotype plasticity (Furness *et al.*, 2015). Others (*e.g.* Miner *et al.*, 2005) highlight the effect of phenotype plasticity on community structure due to phenotypic alteration, in addition to direct and indirect effects of phenotype plasticity with respect to adaptability and alterations in consumption pattern. In this thesis, it

was found that utilisation of sole and enriched substrates by the two Gram-negative strains was impaired by blue light, but to a much lower extent for *S. griseoviridis* and no effect of light regime on substrate utilisation by *B. thuringiensis* was observed. These interactions and the effects of light on substrate utilisation and phenotype plasticity of any of our selected bacterial strains have not been studied previously, but the present findings supported our hypothesis. Thus it might be tempting to draw the conclusion that light spectrum affects the phenotype plasticity of phyllosphere residents, but at the same time it should be keep in mind that of course this does not apply to all epiphytic bacteria.

Light sensing as a signal for morphogenesis and the metabolites for this process are well-known for many bacteria. Previous studies have also reported that non-phototrophic bacteria can sense light, with blue light having an impact on their lifestyle (Kraiselburd *et al.*, 2012; Ondrusch & Kreft, 2011; Swartz *et al.*, 2007; van der Horst *et al.*, 2007). As reported in previous reviews (Losi & Gärtner, 2016; Yin *et al.*, 2013), sensitivity of microbial cells to blue light has been found. In this thesis, putative blue light receptor proteins were found (only two Gram-negative strains were checked). However, knowledge of molecular mechanisms for these selected model species is currently lacking. Based on the results, one could draw the conclusion that blue light mediates substrate utilisation under sole and enriched substrate conditions.

In the selected strains there were indications of light receptor activity, and consequently of monitoring environmental fluctuations. Based on available literature and the present thesis, it might be possible to draw the conclusion that blue light receptors affect metabolic pathways in terms of affecting bacterial lifestyle (biofilm formation, swarming activity *etc*.). This cascade of events will eventually lead to the development of novel strategies to fight bacterial pathogenesis. However, another aspect is that the two *Pseudomonas* species in this thesis harboured regions for putative blue light receptor protein, but some deviations were still observed between their utilisation of sole and enriched substrates on exposure to different light regimes. This shows the importance of nutritional status, beside light receptors, an important topic for further analyses and future studies.

3.3 Impact of light spectrum on the formation of metabolites decisive for leaf colonisation (Paper IV)

Biosurfactants are a group of diverse molecules that have several biological function (Banat *et al.*, 2010). Biosurfactants share a basic structure, which

consists of a hydrophobic and a hydrophilic moiety (Mulligan, 2005). The nature of biosurfactants is to produce amphiphilic compounds that exhibit surface activity (Burch *et al.*, 2011). These molecules act in three ways: (i) adaptation of surface properties (Debode *et al.*, 2007), (ii) alteration of compound bio-availability (Das & Mukherjee, 2007) and (iii) interaction with membranes (de Bruijn *et al.*, 2007). Their action is a function of their specific structure and production characteristics (D'aes *et al.*, 2010)

Microorganisms that are able to form biosurfactants can be found in every imaginable environment (Kefi *et al.*, 2015; Hultberg *et al.*, 2010; Haddad *et al.*, 2008). The frequency and versatility of surface-active compounds produced displays the importance of biosurfactants for the functioning and survival of the organisms (Banat *et al.*, 2010). Epiphytic bacteria utilise biosurfactants to increase the wettability of the leaf, to increase nutrient transmission across the waxy cuticle and to move to favourable growth sites (D'aes *et al.*, 2010; Lindow & Brandl, 2003). The many possible contributions to epiphytic fitness of biosurfactant-producing bacteria inspired the work in this thesis to check the capacity of four selected bacterial strains to form biosurfactants as a response to nutrient availability and light spectrum.

In Paper IV, the biosurfactant formation of four strains was evaluated using the drop collapse test (see Materials & Methods section in Paper IV). The results indicated that the effect of light regime on biosurfactant production was lower and inconsistent for the two Gram-positive strains studied. However, the two Gram-negative strains produced biosurfactants when exposed to different light treatments. Biosurfactant production was decreased when the bacteria were grown under a blue LED spectrum.

Another interesting finding in this thesis was that the four strains examined behaved differently when utilising different substrates (Tween 20, Tween 40 and Tween 80), which themselves are surfactant agents. Moreover, observations of drop collapse on these surfactant substrates even after exposure to different light spectra and of different utilisation patterns on these substrates as a function of light regime indicate an impact of light spectrum on biosurfactant formation. It was also found that, besides effects of light spectrum, substrate richness was decisive for biosurfactant formation. For instance D-alanine, L-phenyl alanine, glycine, L-threonine, α-amino-N valeric acid and γ-amino-N-valeric acid, when supplied as N source, were utilised and affected the capacity of *P. agarici* to form biosurfactants, but not when supplied as C source. However, for other substrates such as iso-leucine, biosurfactant was formed in both sole and enriched substrate when exposed to blue LED light.

Other strains of the four species examined in this thesis have already been reported to display a capacity to form biosurfactants (Silva *et al.*, 2016; Kefi *et al.*, 2015; Kalyani *et al.*, 2014; Hue *et al.*, 2001), and tested positive for this property in Paper II when screened *in vitro*. As there is little published information regarding biosurfactant formation by these selected strains and the chemical nature of compound/s behind this activity, it is difficult to draw firm conclusion on which compounds are decisive for biosurfactant formation.

In a plant ecology perspective, the role of biosurfactants is different (Hultberg *et al.*, 2010; Raaijmakers *et al.*, 2006). For instance, biosurfactants might promote bacterial colonisation of leaves to find new habitats in the phyllosphere, or reduce the tension of the leaf surface caused by the waxy layer of leaf cuticle. The findings in Paper IV suggest that the ability to investigate new habitats in the plant phyllosphere is a function of both light spectrum and presence of certain precursor compounds.

Another interesting finding in this thesis was that, under blue LED spectrum biosurfactant formation was not supported in the presence of some enriched substrates. This might indicate that the blue light spectrum and nutritional factors affected bacterial ability to form biosurfactants, possibly through a switch in metabolic pathway. Previous studies have reported that blue and white light inhibit the swarming motility of *Pseudomonas syringae* pv tomato DC3000 (Río-Álvarez *et al.*, 2014), and regulate the swarming motility of the foliar pathogen *Pseudomonas syringae* (Wu *et al.*, 2013). Blue light has also antimicrobial activity against many bacterial and fungal pathogens (Bonomi *et al.*, 2016; El Din *et al.*, 2016; Ricci *et al.*, 2015; Maclean *et al.*, 2014). All these findings were supported by the results in this thesis. Together, these findings raise the following questions: (i). How can new approaches with respect to lighting strategy be exploited for better establishment of *Pseudomonas* biocontrol strains? and (ii) How can light treatment be used to suppress the impact of plant pathogenic bacteria?

4 Conclusions

The key findings of this thesis were that:

- Choice of light for artificial assimilation lighting in the greenhouse can affect sunflower plant shape and its phyllosphere microbial community structure.

- Several dominant bacterial leaf colonisers are dependent on leaf temperature.

- Light source affects phyllosphere-associated fungi (directly through the physical properties of the chosen light source) and bacteria (indirectly through alterations in the plant environment) on sunflower leaves.

- Complementary use of culture-dependent and culture-independent methods is a suitable approach for investigating microbial communities in the phyllosphere of greenhouse-grown ornamentals.

- There is a need for standard protocols to survey the phyllosphere of greenhouse-grown ornamentals when mining for microbial biocontrol agents with the potential to control diseases.

- Phenotypic properties of microorganisms may change when greenhouse-grown ornamentals are exposed to artificial lighting.

- Interactions between light and phyllosphere microbiota are very complex. Therefore, properties of the light supplied need to be considered when measuring beneficial, neutral and deleterious microbial effects.

- The phenotypic array technique method developed in this thesis can be used to identify the different metabolic pathways influenced by light spectra and to predict microbial responses to different light spectra.

- Ability of epiphytic phyllosphere colonisers to investigate new habitats in the plant phyllosphere is a function of both light spectrum and the presence of certain precursor compounds.

References

Ávila-Pérez, M., Hellingwerf, K.J. & Kort, R. (2006). Blue light activates the σB-dependent stress response of Bacillus subtilis via YtvA. *Journal of bacteriology*, 188(17), pp. 6411-6414.

Bálint, M., Bartha, L., O'Hara, R.B., Olson, M.S., Otte, J., Pfenninger, M., Robertson, A.L., Tiffin, P. & Schmitt, I. (2015). Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular ecology*, 24(1), pp. 235-248.

Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J. & Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. *Applied microbiology and biotechnology*, 87(2), pp. 427-444.

Beattie, G., Lindow, S., Hecht-Poinar, E. & Elliott, V. (2002). Leaf surface waxes and the process of leaf colonization by microorganisms. *Phyllosphere microbiology*, pp. 3-26.

Beier, S., Rivers, A.R., Moran, M.A. & Obernosterer, I. (2015). Phenotypic plasticity in heterotrophic marine microbial communities in continuous cultures. *The ISME journal*, 9(5), pp. 1141-1151.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A. & Hallmann, J. (2005). Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiology Ecology*, 51(2), pp. 215-229.

Bergstrand, K.-J. & Schüssler, H. Recent progresses on the application of LEDs in the horticultural production. In: *Proceedings of XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 927*2010, pp. 529-534.

Bernard, F., Sache, I., Suffert, F. & Chelle, M. (2013). The development of a foliar fungal pathogen does react to leaf temperature! *New Phytologist*, 198(1), pp. 232-240.

Besnard, V., Federighi, M. & Cappelier, J. (2000). Development of a direct viable count procedure for the investigation of VBNC state in Listeria monocytogenes. *Letters in applied Microbiology*, 31(1), pp. 77-81.

Bian, Z.H., Yang, Q.C. & Liu, W.K. (2015). Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. *Journal of the Science of Food and Agriculture*, 95(5), pp. 869-877.

Bochner, B.R. (2003). New technologies to assess genotype–phenotype relationships. *Nature Reviews Genetics*, 4(4), pp. 309-314.

Bochner, B.R. (2009). Global phenotypic characterization of bacteria. *FEMS microbiology reviews*, 33(1), pp. 191-205.

Bochner, B.R., Gadzinski, P. & Panomitros, E. (2001). Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. *Genome research*, 11(7), pp. 1246-1255.

Bodenhausen, N., Bortfeld-Miller, M., Ackermann, M. & Vorholt, J.A. (2014). A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLoS Genet*, 10(4), p. e1004283.

Bonomi, H.R., Toum, L., Sycz, G., Sieira, R., Toscani, A.M., Gudesblat, G.E., Leskow, F.C., Goldbaum, F.A., Vojnov, A.A. & Malamud, F. (2016). Xanthomonas campestris attenuates virulence by sensing light through a bacteriophytochrome photoreceptor. *EMBO reports*, p. e201541691.

Bourget, C.M. (2008). An introduction to light-emitting diodes. *HortScience*, 43(7), pp. 1944-1946.

Bradshaw, A.D. (1965). Evolutionary significance of phenotypic plasticity in plants. *Advances in genetics*, 13, pp. 115-155.

Brandl, M.T. & Mandrell, R.E. (2002). Fitness of Salmonella enterica serovar Thompson in the cilantro phyllosphere. *Applied and environmental microbiology*, 68(7), pp. 3614-3621.

Braun, U. (1995). *The powdery mildews (Erysiphales) of Europe*: VEB Gustav Fischer Verlag.

Brazaityte, A., Ulinskaite, R., Duchovskis, P., Samuoliene, G., Siksnianiene, J., Jankauskiene, J., Sabajeviene, G., Baranauskis, K., Staniene, G. & Tamulaitis, G. (2006). Optimization of lighting spectrum for photosynthetic system and productivity of lettuce by using light-emitting diodes. *Acta Horticulturae*, 711, p. 183.

Bula, R., Morrow, R., Tibbitts, T., Barta, D., Ignatius, R. & Martin, T. (1991). Light-emitting diodes as a radiation source for plants. *HortScience*, 26(2), pp. 203-205.

Bunster, L., Fokkema, N.J. & Schippers, B. (1989). Effect of surface-active Pseudomonas spp. on leaf wettability. *Applied and environmental microbiology*, 55(6), pp. 1340-1345.

Burch, A.Y., Browne, P.J., Dunlap, C.A., Price, N.P. & Lindow, S.E. (2011). Comparison of biosurfactant detection methods reveals hydrophobic surfactants and contact-regulated production. *Environmental Microbiology*, 13(10), pp. 2681-2691.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L. & Bauer, M. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal*, 6(8), pp. 1621-1624.

Carlsson-Kanyama, A. (1998). Food consumption patterns and their influence on climate change: greenhouse gas emissions in the life-cycle of tomatoes and carrots consumed in Sweden. *Ambio*, pp. 528-534.

Carvalho, S.D. & Folta, K.M. (2014). Sequential light programs shape kale (Brassica napus) sprout appearance and alter metabolic and nutrient content. *Horticulture research*, 1, p. 8.

Chen, M., Chory, J. & Fankhauser, C. (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.*, 38, pp. 87-117.

Chen, R.-S., Chu, C., Cheng, C.-W., Chen, W.-Y. & Tsay, J.-G. (2008). Differentiation of two powdery mildews of sunflower (Helianthus annuus) by a PCR-mediated method based on ITS sequences. *European journal of plant pathology*, 121(1), pp. 1-8.

Chen, X., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J. & Borriss, R. (2009). Genome analysis of Bacillus amyloliquefaciens FZB42 reveals its potential for biocontrol of plant pathogens. *Journal of biotechnology*, 140(1), pp. 27-37.

Collier, D., Hager, P. & Phibbs, P. (1996). Catabolite repression control in the Pseudomonads. *Research in microbiology*, 147(6), pp. 551-561.

D'aes, J., De Maeyer, K., Pauwelyn, E. & Höfte, M. (2010). Biosurfactants in plant–Pseudomonas interactions and their importance to biocontrol. *Environmental microbiology reports*, 2(3), pp. 359-372.

Das, K. & Mukherjee, A. (2007). Differential utilization of pyrene as the sole source of carbon by Bacillus subtilis and Pseudomonas aeruginosa strains: role of biosurfactants in enhancing bioavailability. *Journal of applied microbiology*, 102(1), pp. 195-203.

Davis, C. & Brlansky, R. (1991). Use of immunogold labelling with scanning electron microscopy to identify phytopathogenic bacteria on leaf surfaces. *Applied and environmental microbiology*, 57(10), pp. 3052-3055.

de Bruijn, I., de Kock, M.J., Yang, M., de Waard, P., van Beek, T.A. & Raaijmakers, J.M. (2007). Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. *Molecular microbiology*, 63(2), pp. 417-428.

de Carbonnel, M., Davis, P., Roelfsema, M.R.G., Inoue, S.-i., Schepens, I., Lariguet, P., Geisler, M., Shimazaki, K.-i., Hangarter, R. & Fankhauser, C. (2010). The Arabidopsis PHYTOCHROME KINASE SUBSTRATE2 protein is a phototropin signaling element that regulates leaf flattening and leaf positioning. *Plant physiology*, 152(3), pp. 1391-1405.

Dean, R., Van Kan, J.A., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R. & Ellis, J. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular plant pathology*, 13(4), pp. 414-430.

Debode, J., Maeyer, K.D., Perneel, M., Pannecoucque, J., Backer, G.D. & Höfte, M. (2007). Biosurfactants are involved in the biological control of Verticillium microsclerotia by Pseudomonas spp. *Journal of applied microbiology*, 103(4), pp. 1184-1196.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C. & Vorholt, J.A. (2009). Community proteogenomics

reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences*, 106(38), pp. 16428-16433.
Devlin, P.F., Christie, J.M. & Terry, M.J. (2007). Many hands make light work. *Journal of experimental botany*, 58(12), pp. 3071-3077.
Ding, T. & Melcher, U. (2016). Influences of plant species, season and location on leaf endophytic bacterial communities of non-cultivated plants. *PloS one*, 11(3), p. e0150895.
Ding, T., Palmer, M.W. & Melcher, U. (2013). Community terminal restriction fragment length polymorphisms reveal insights into the diversity and dynamics of leaf endophytic bacteria. *BMC microbiology*, 13(1), p. 1.
Downs, R.J. & Thomas, J.F. (1982). Phytochrome regulation of flowering in the long-day plant, Hyoscyamus niger. *Plant physiology*, 70(3), pp. 898-900.
El Din, S.N., El-Tayeb, T.A., Abou-Aisha, K. & El-Azizi, M. (2016). In vitro and in vivo antimicrobial activity of combined therapy of silver nanoparticles and visible blue light against Pseudomonas aeruginosa. *International journal of nanomedicine*, 11, p. 1749.
Fankhauser, C. & Chory, J. (1997). Light control of plant development. *Annual review of cell and developmental biology*, 13(1), pp. 203-229.
Folta, K.M. & Childers, K.S. (2008). Light as a growth regulator: controlling plant biology with narrow-bandwidth solid-state lighting systems. *HortScience*, 43(7), pp. 1957-1964.
Foster, K.R. & Bell, T. (2012). Competition, not cooperation, dominates interactions among culturable microbial species. *Current biology*, 22(19), pp. 1845-1850.
Fukuda, N., Fujita, M., Ohta, Y., Sase, S., Nishimura, S. & Ezura, H. (2008). Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. *Scientia Horticulturae*, 115(2), pp. 176-182.
Furness, A.I., Lee, K. & Reznick, D.N. (2015). Adaptation in a variable environment: Phenotypic plasticity and bet-hedging during egg diapause and hatching in an annual killifish. *Evolution*, 69(6), pp. 1461-1475.
Gadtke, L. (2010). Denmark-Sustainable horticulture crop production.
Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional ecology*, 21(3), pp. 394-407.
Gomelsky, M. & Hoff, W.D. (2011). Light helps bacteria make important lifestyle decisions. *Trends in microbiology*, 19(9), pp. 441-448.
Görke, B. & Stülke, J. (2008). Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nature Reviews Microbiology*, 6(8), pp. 613-624.
Haddad, N.I., Wang, J. & Mu, B. (2008). Isolation and characterization of a biosurfactant producing strain, Brevibacilis brevis HOB1. *Journal of industrial microbiology & biotechnology*, 35(12), pp. 1597-1604.
Hahn, M. (2014). The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. *Journal of chemical biology*, 7(4), pp. 133-141.

Hakovirta, J.R., Prezioso, S., Hodge, D., Pillai, S.P. & Weigel, L.M. (2016). Identification and analysis of informative single nucleotide polymorphisms in 16S rRNA gene sequences of the Bacillus cereus group. *Journal of Clinical Microbiology*, 54(11), pp. 2749-2756.

Heuvelink, E., Bakker, M., Hogendonk, L., Janse, J., Kaarsemaker, R. & Maaswinkel, R. Horticultural lighting in the Netherlands: new developments. In: *Proceedings of V International Symposium on Artificial Lighting in Horticulture 711*2006, pp. 25-34.

Higgins, C.F. (1992). ABC transporters: from microorganisms to man. *Annual review of cell biology*, 8(1), pp. 67-113.

Hodkinson, B.P. & Grice, E.A. (2015). Next-generation sequencing: a review of technologies and tools for wound microbiome research. *Advances in wound care*, 4(1), pp. 50-58.

Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W. & Harbinson, J. (2010). Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. *Journal of experimental botany*, p. erq132.

Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S. & Leduc, N. (2015). Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. *Environmental and Experimental Botany*, 121, pp. 22-38.

Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S. & Leduc, N. (2016). Light signaling and plant responses to blue and UV radiations—Perspectives for applications in horticulture. *Environmental and Experimental Botany*, 121, pp. 22-38.

Hue, N., Serani, L. & Laprévote, O. (2001). Structural investigation of cyclic peptidolipids from Bacillus subtilis by high-energy tandem mass spectrometry. *Rapid communications in mass spectrometry*, 15(3), pp. 203-209.

Hultberg, M., Alsberg, T., Khalil, S. & Alsanius, B. (2010). Suppression of disease in tomato infected by Pythium ultimum with a biosurfactant produced by Pseudomonas koreensis. *BioControl*, 55(3), pp. 435-444.

Hunter, P.J., Hand, P., Pink, D., Whipps, J.M. & Bending, G.D. (2010). Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (Lactuca species) phyllosphere. *Applied and environmental microbiology*, 76(24), pp. 8117-8125.

Hussa, E.A. & Goodrich-Blair, H. (2013). It takes a village: ecological and fitness impacts of multipartite mutualism. *Annual review of microbiology*, 67, pp. 161-178.

Itagaki, K., Shibuya, T., Tojo, M., Endo, R. & Kitaya, Y. (2016). Early Development of Powdery Mildew on Cucumber Leaves Acclimatized to Illumination with Different Red-to-far-red Ratios. *HortScience*, 51(5), pp. 530-536.

Jager, E., Wehner, F. & Korsten, L. (2001). Microbial ecology of the mango phylloplane. *Microbial ecology*, 42(2), pp. 201-207.

Jensen, B., Knudsen, I.M., Andersen, B., Nielsen, K.F., Thrane, U., Jensen, D.F. & Larsen, J. (2013). Characterization of microbial communities and fungal metabolites on field grown strawberries from organic and conventional production. *International journal of food microbiology,* 160(3), pp. 313-322.

Johkan, M., Shoji, K., Goto, F., Hahida, S. & Yoshihara, T. (2012). Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in Lactuca sativa. *Environmental and Experimental Botany,* 75, pp. 128-133.

Johkan, M., Shoji, K., Goto, F., Hashida, S.-n. & Yoshihara, T. (2010). Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience,* 45(12), pp. 1809-1814.

Joshi, J.R., Burdman, S., Lipsky, A. & Yedidia, I. (2015). Effects of plant antimicrobial phenolic compounds on virulence of the genus Pectobacterium. *Research in microbiology,* 166(6), pp. 535-545.

Kalyani, A., Naga Sireesha, G., Aditya, A., Girija Sankar, G. & Prabhakar, T. (2014). Production Optimization of Rhamnolipid Biosurfactant by Streptomyces coelicoflavus (NBRC 15399T) using Plackett-Burman design. *Eur J Biotechno l Biosci,* 1, pp. 07-13.

Kefi, A., Slimene, I.B., Karkouch, I., Rihouey, C., Azaeiz, S., Bejaoui, M., Belaid, R., Cosette, P., Jouenne, T. & Limam, F. (2015). Characterization of endophytic Bacillus strains from tomato plants (Lycopersicon esculentum) displaying antifungal activity against Botrytis cinerea Pers. *World Journal of Microbiology and Biotechnology,* 31(12), pp. 1967-1976.

Kemen, E. (2014). Microbe–microbe interactions determine oomycete and fungal host colonization. *Current opinion in plant biology,* 20, pp. 75-81.

Kim, H.-S., Sang, M.K., Jung, H.W., Jeun, Y.-C., Myung, I.-S. & Kim, K.D. (2012). Identification and characterization of Chryseobacterium wanjuense strain KJ9C8 as a biocontrol agent of Phytophthora blight of pepper. *Crop Protection,* 32, pp. 129-137.

Kim, J.J. & Sundin, G.W. (2000). Regulation of the rulAB mutagenic DNA repair operon of Pseudomonas syringae by UV-B (290 to 320 nanometers) radiation and analysis of rulAB-mediated mutability in vitro and in planta. *Journal of bacteriology,* 182(21), pp. 6137-6144.

Kinkel, L., Wilson, M. & Lindow, S. (2000). Plant species and plant incubation conditions influence variability in epiphytic bacterial population size. *Microbial ecology,* 39(1), pp. 1-11.

Kinkel, L.L. (1997). Microbial population dynamics on leaves. *Annual review of phytopathology,* 35(1), pp. 327-347.

Knief, C., Ramette, A., Frances, L., Alonso-Blanco, C. & Vorholt, J.A. (2010). Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. *The ISME journal,* 4(6), pp. 719-728.

Kong, S.-G. & Okajima, K. (2016). Diverse photoreceptors and light responses in plants. *Journal of plant research,* 129(2), pp. 111-114.

Kopsell, D.A. & Sams, C.E. (2013). Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *Journal of the American society for horticultural science,* 138(1), pp. 31-37.

Kraiselburd, I., Alet, A.I., Tondo, M.L., Petrocelli, S., Daurelio, L.D., Monzón, J., Ruiz, O.A., Losi, A. & Orellano, E.G. (2012). A LOV protein modulates the physiological attributes of Xanthomonas axonopodis pv. citri relevant for host plant colonization. *PloS one,* 7(6), p. e38226.

Laforest-Lapointe, I., Messier, C. & Kembel, S.W. (2016). Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome,* 4(1), p. 27.

Lambais, M.R., Lucheta, A.R. & Crowley, D.E. (2014). Bacterial community assemblages associated with the phyllosphere, dermosphere, and rhizosphere of tree species of the Atlantic forest are host taxon dependent. *Microbial ecology,* 68(3), pp. 567-574.

Lattanzio, V., Lattanzio, V.M. & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in research,* 661, pp. 23-67.

Li, Q. & Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany,* 67(1), pp. 59-64.

Li, S.-B., Fang, M., Zhou, R.-C. & Huang, J. (2012). Characterization and evaluation of the endophyte Bacillus B014 as a potential biocontrol agent for the control of Xanthomonas axonopodis pv. dieffenbachiae–Induced blight of Anthurium. *Biological Control,* 63(1), pp. 9-16.

Li, W. & Lu, C.-D. (2007). Regulation of carbon and nitrogen utilization by CbrAB and NtrBC two-component systems in Pseudomonas aeruginosa. *Journal of bacteriology,* 189(15), pp. 5413-5420.

Lin, K.-H., Huang, M.-Y., Huang, W.-D., Hsu, M.-H., Yang, Z.-W. & Yang, C.-M. (2013). The effects of red, blue, and white light-emitting diodes on the growth, development, and edible quality of hydroponically grown lettuce (Lactuca sativa L. var. capitata). *Scientia Horticulturae,* 150, pp. 86-91.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S. & Stenlid, J. (2013). Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytologist,* 199(1), pp. 288-299.

Lindow, S.E. & Brandl, M.T. (2003). Microbiology of the phyllosphere. *Applied and environmental microbiology,* 69(4), pp. 1875-1883.

Line, J., Hiett, K., Guard, J. & Seal, B. (2011). Temperature affects sole carbon utilization patterns of Campylobacter coli 49941. *Current microbiology,* 62(3), pp. 821-825.

Lopez-Velasco, G., Tydings, H.A., Boyer, R.R., Falkinham, J.O. & Ponder, M.A. (2012). Characterization of interactions between Escherichia coli O157: H7 with epiphytic bacteria in vitro and on spinach leaf surfaces. *International journal of food microbiology,* 153(3), pp. 351-357.

Losi, A. (2004). The bacterial counterparts of plant phototropins. *Photochemical & Photobiological Sciences,* 3(6), pp. 566-574.

Losi, A. & Gärtner, W. (2016). Solving Blue-light Riddles: New Lessons from Flavin-binding LOV Photoreceptors. *Photochemistry and Photobiology*.

Luo, C., Tsementzi, D., Kyrpides, N., Read, T. & Konstantinidis, K.T. (2012). Direct comparisons of Illumina vs. Roche 454 sequencing technologies on the same microbial community DNA sample. *PloS one*, 7(2), p. e30087.

Macedo, A.F., Leal-Costa, M.V., Tavares, E.S., Lage, C.L.S. & Esquibel, M.A. (2011). The effect of light quality on leaf production and development of in vitro-cultured plants of Alternanthera brasiliana Kuntze. *Environmental and Experimental Botany*, 70(1), pp. 43-50.

Maclean, M., McKenzie, K., Anderson, J.G., Gettinby, G. & MacGregor, S.J. (2014). 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. *Journal of Hospital Infection*, 88(1), pp. 1-11.

Madigan, M., Martinko, J., Stahl, D. & Clark, D. (2012). Brock Biology of Microorganisms.(13 Eth) Pearson Education. *San Francisco, USA*.

Maqbool, A., Horler, R.S., Muller, A., Wilkinson, A.J., Wilson, K.S. & Thomas, G.H. (2015). The substrate-binding protein in bacterial ABC transporters: dissecting roles in the evolution of substrate specificity. *Biochemical Society Transactions*, 43(5), pp. 1011-1017.

Marcelis, L., Elings, A., Bakker, M., Brajeul, E., Dieleman, J., De Visser, P. & Heuvelink, E. Modelling dry matter production and partitioning in sweet pepper. In: *Proceedings of III International Symposium on Models for Plant Growth, Environmental Control and Farm Management in Protected Cultivation 718*2006, pp. 121-128.

Mardis, E.R. (2008). The impact of next-generation sequencing technology on genetics. *Trends in genetics*, 24(3), pp. 133-141.

Mariano, R. & McCarter, S. (1993). Epiphytic survival of Pseudomonas viridiflava on tomato and selected weed species. *Microbial ecology*, 26(1), pp. 47-58.

Mason, C.J., Pfammatter, J.A., Holeski, L.M. & Raffa, K.F. (2014). Foliar bacterial communities of trembling aspen in a common garden. *Canadian Journal of Microbiology*, 61(2), pp. 143-149.

Massa, G.D., Kim, H.-H., Wheeler, R.M. & Mitchell, C.A. (2008). Plant productivity in response to LED lighting. *HortScience*, 43(7), pp. 1951-1956.

Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K. & Relyea, R.A. (2005). Ecological consequences of phenotypic plasticity. *Trends in Ecology & Evolution*, 20(12), pp. 685-692.

Mitchell, C.A., Both, A.-J., Bourget, C., Burr, J., Kubota, C., Lopez, R., Morrow, R. & Runkle, E. (2012). Horticultural Science Focus-LEDs: The Future of Greenhouse Lighting! *Chronica Horticulturae-Subscription*, 52(1), p. 6.

Mole, B. (2013). Microbiome research goes without a home. *Nature*, 500(7460), pp. 16-16.

Moreno, R., Martínez-Gomariz, M., Yuste, L., Gil, C. & Rojo, F. (2009). The Pseudomonas putida Crc global regulator controls the hierarchical assimilation of amino acids in a complete medium: evidence from proteomic and genomic analyses. *Proteomics*, 9(11), pp. 2910-2928.

Morey, M., Fernández-Marmiesse, A., Castiñeiras, D., Fraga, J.M., Couce, M.L. & Cocho, J.A. (2013). A glimpse into past, present, and future DNA sequencing. *Molecular genetics and metabolism*, 110(1), pp. 3-24.

Morris, C., Kinkel, L., Lindow, S., Hecht-Poinar, E. & Elliott, V. (2002). Fifty years of phyllosphere microbiology: significant contributions to research in related fields. *Phyllosphere microbiology*, pp. 365-375.

Morris, C.E. & Monier, J.-M. (2003). The ecological significance of biofilm formation by plant-associated bacteria. *Annual review of phytopathology*, 41(1), pp. 429-453.

Morrow, R.C. (2008). LED lighting in horticulture. *HortScience*, 43(7), pp. 1947-1950.

Mulligan, C.N. (2005). Environmental applications for biosurfactants. *Environmental pollution*, 133(2), pp. 183-198.

Mulpuri, S., Soni, P.K. & Gonela, S.K. (2016). Morphological and molecular characterization of powdery mildew on sunflower (Helianthus annuus L.), alternate hosts and weeds commonly found in and around sunflower fields in India. *Phytoparasitica*, 44(3), pp. 353-367.

Nishio, J. (2000). Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant, Cell & Environment*, 23(6), pp. 539-548.

Nocker, A., Burr, M. & Camper, A.K. (2007). Genotypic microbial community profiling: a critical technical review. *Microbial ecology*, 54(2), pp. 276-289.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K. & Kurata, K. (2007). Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. *Environmental Control in Biology*, 45(3), pp. 189-198.

Ondrusch, N. & Kreft, J. (2011). Blue and red light modulates SigB-dependent gene transcription, swimming motility and invasiveness in Listeria monocytogenes. *PloS one*, 6(1), p. e16151.

Onstott, T., Phelps, T.J., Colwell, F., Ringelberg, D., White, D., Boone, D., Mckinley, J.P., Stevens, T.O., Long, P.E. & Balkwill, D. (1998). Observations pertaining to the origin and ecology of microorganisms recovered from the deep subsurface of Taylorsville Basin, Virginia. *Geomicrobiology Journal*, 15(4), pp. 353-385.

Opdam, J., Schoonderbeek, G., Heller, E. & De Gelder, A. Closed greenhouse: a starting point for sustainable entrepreneurship in horticulture. In: *Proceedings of International Conference on Sustainable Greenhouse Systems-Greensys2004 691*2004, pp. 517-524.

Opdam, J., Schoonderbeek, G., Heller, E. & De Gelder, A. Closed greenhouse: a starting point for sustainable entrepreneurship in horticulture. In: *Proceedings of International Conference on Sustainable Greenhouse Systems-Greensys2004 691*2005, pp. 517-524.

Ouzounis, T., Rosenqvist, E. & Ottosen, C.-O. (2015). Spectral effects of artificial light on plant physiology and secondary metabolism: a review. *HortScience*, 50(8), pp. 1128-1135.

Paradiso, R., Meinen, E., Snel, J.F., De Visser, P., Van Ieperen, W., Hogewoning, S.W. & Marcelis, L.F. (2011). Spectral dependence of photosynthesis and

light absorptance in single leaves and canopy in rose. *Scientia Horticulturae*, 127(4), pp. 548-554.

Penuelas, J., Rico, L., Ogaya, R., Jump, A. & Terradas, J. (2012). Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of Quercus ilex in a mixed Mediterranean forest. *Plant Biology*, 14(4), pp. 565-575.

Purcell, E.B., Siegal-Gaskins, D., Rawling, D.C., Fiebig, A. & Crosson, S. (2007). A photosensory two-component system regulates bacterial cell attachment. *Proceedings of the National Academy of Sciences*, 104(46), pp. 18241-18246.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic acids research*, 41(D1), pp. D590-D596.

Raaijmakers, J.M., de Bruijn, I. & de Kock, M.J. (2006). Cyclic lipopeptide production by plant-associated Pseudomonas spp.: diversity, activity, biosynthesis, and regulation. *Molecular Plant-Microbe Interactions*, 19(7), pp. 699-710.

Rastogi, G., Coaker, G.L. & Leveau, J.H. (2013). New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS microbiology letters*, 348(1), pp. 1-10.

Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y. & Fierer, N. (2010). The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology*, 12(11), pp. 2885-2893.

Redford, A.J. & Fierer, N. (2009). Bacterial succession on the leaf surface: a novel system for studying successional dynamics. *Microbial ecology*, 58(1), pp. 189-198.

Rees, D.C., Johnson, E. & Lewinson, O. (2009). ABC transporters: the power to change. *Nature reviews Molecular cell biology*, 10(3), pp. 218-227.

Reisberg, E.E., Hildebrandt, U., Riederer, M. & Hentschel, U. (2012). Phyllosphere bacterial communities of trichome-bearing and trichomeless Arabidopsis thaliana leaves. *Antonie Van Leeuwenhoek*, 101(3), pp. 551-560.

Ricci, A., Dramis, L., Shah, R., Gärtner, W. & Losi, A. (2015). Visualizing the relevance of bacterial blue-and red-light receptors during plant–pathogen interaction. *Environmental microbiology reports*, 7(5), pp. 795-802.

Rigonato, J., Gonçalves, N., Andreote, A.P.D., Lambais, M.R. & Fiore, M.F. (2016). Estimating genetic structure and diversity of cyanobacterial communities in Atlantic forest phyllosphere. *Canadian Journal of Microbiology*, 62(11), pp. 953-960.

Río-Álvarez, I., Rodríguez-Herva, J.J., Martínez, P.M., González-Melendi, P., García-Casado, G., Rodríguez-Palenzuela, P. & López-Solanilla, E. (2014). Light regulates motility, attachment and virulence in the plant pathogen Pseudomonas syringae pv tomato DC3000. *Environmental Microbiology*, 16(7), pp. 2072-2085.

Rojo, F. (2010). Carbon catabolite repression in Pseudomonas: optimizing metabolic versatility and interactions with the environment. *FEMS microbiology reviews*, 34(5), pp. 658-684.

Romero, D., De Vicente, A., Olmos, J., Davila, J. & Pérez-García, A. (2007). Effect of lipopeptides of antagonistic strains of Bacillus subtilis on the morphology and ultrastructure of the cucurbit fungal pathogen Podosphaera fusca. *Journal of applied microbiology*, 103(4), pp. 969-976.

Runkle, E.S. & Heins, R.D. (2001). Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. *Journal of the American society for horticultural science*, 126(3), pp. 275-282.

Rupp, S., Weber, R.W., Detzel, P., Rieger, D. & Hahn, M. (2016). Spread of Botrytis cinerea strains with multiple fungicide resistance in German horticulture. *Frontiers in Microbiology*, 7, p. 2075.

Salinas, S. & Munch, S.B. (2012). Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecology Letters*, 15(2), pp. 159-163.

Sandhu, A., Halverson, L.J. & Beattie, G.A. (2007). Bacterial degradation of airborne phenol in the phyllosphere. *Environmental Microbiology*, 9(2), pp. 383-392.

Schreiber, L., Krimm, U., Knoll, D., Sayed, M., Auling, G. & Kroppenstedt, R.M. (2005). Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. *New Phytologist*, 166(2), pp. 589-594.

Schubert, E.F. & Kim, J.K. (2005). Solid-state light sources getting smart. *Science*, 308(5726), pp. 1274-1278.

Schuerger, A. & Brown, C. (1994). Spectral quality may be used to alter plant disease development in CELSS. *Advances in Space Research*, 14(11), pp. 395-398.

Schuerger, A.C., Brown, C.S. & Stryjewski, E.C. (1997). Anatomical features of pepper plants (Capsicum annuum L.) grown under red light-emitting diodes supplemented with blue or far-red light. *Annals of Botany*, 79(3), pp. 273-282.

Shade, A., Hogan, C.S., Klimowicz, A.K., Linske, M., McManus, P.S. & Handelsman, J. (2012). Culturing captures members of the soil rare biosphere. *Environmental Microbiology*, 14(9), pp. 2247-2252.

Silva, M., Silva, A.F., Rufino, R.D., Luna, J.M., Santos, V.A. & Sarubbo, L.A. (2016). Production of Biosurfactants by Pseudomonas Species for Application in the Petroleum Industry. *Water environment research: a research publication of the Water Environment Federation*.

Singh, D., Basu, C., Meinhardt-Wollweber, M. & Roth, B. (2015). LEDs for energy efficient greenhouse lighting. *Renewable and Sustainable Energy Reviews*, 49, pp. 139-147.

Sinha, R.P. & Häder, D.-P. (2002). UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences*, 1(4), pp. 225-236.

Siqueira, J.F., Fouad, A.F. & Roˆcas, I.N. (2012). Pyrosequencing as a tool for better understanding of human microbiomes. *Journal of oral microbiology*, 4.

Skidmore, A. & Dickinson, C. (1976). Colony interactions and hyphal interference between Septoria nodorum and phylloplane fungi. *Transactions of the British Mycological Society*, 66(1), pp. 57-64.

Soberón-Chávez, G., Lépine, F. & Déziel, E. (2005). Production of rhamnolipids by Pseudomonas aeruginosa. *Applied microbiology and biotechnology*, 68(6), pp. 718-725.

Stadler, B. & Müller, T. (2000). Effects of aphids and moth caterpillars on epiphytic microorganisms in canopies of forest trees. *Canadian journal of forest research*, 30(4), pp. 631-638.

Stefani, F.O., Bell, T.H., Marchand, C., Ivan, E., El Yassimi, A., St-Arnaud, M. & Hijri, M. (2015). Culture-dependent and-independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. *PloS one*, 10(6), p. e0128272.

Stutte, G.W., Edney, S. & Skerritt, T. (2009). Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. *HortScience*, 44(1), pp. 79-82.

Sultan, S.E. & Stearns, S.C. (2005). Environmentally contingent variation: phenotypic plasticity and norms of reaction. *Variation. A Central Concept in Biology*, pp. 303-332.

Sundin, G. & Jacobs, J. (1999). Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (Arachis hypogeae L.). *Microbial ecology*, 38(1), pp. 27-38.

Sundin, G., Lindow, S., Hecht-Poinar, E. & Elliott, V. (2002). Ultraviolet radiation on leaves: its influence on microbial communities and their adaptations. *Phyllosphere microbiology*, pp. 27-41.

Suthaparan, A., Torre, S., Stensvand, A., Herrero, M., Pettersen, R., Gadoury, D. & Gislerød, H. (2010). Specific light-emitting diodes can suppress sporulation of Podosphaera pannosa on greenhouse roses. *Plant disease*, 94(9), pp. 1105-1110.

Swartz, T.E., Tseng, T.-S., Frederickson, M.A., Paris, G., Comerci, D.J., Rajashekara, G., Kim, J.-G., Mudgett, M.B., Splitter, G.A. & Ugalde, R.A. (2007). Blue-light-activated histidine kinases: two-component sensors in bacteria. *Science*, 317(5841), pp. 1090-1093.

Sylla, J., Alsanius, B.W., Krüger, E., Becker, D. & Wohanka, W. (2013). In vitro compatibility of microbial agents for simultaneous application to control strawberry powdery mildew (Podosphaera aphanis). *Crop Protection*, 51, pp. 40-47.

Team, R.C. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2015. URL h ttp. *www. R-project. org*.

Thompson, I., Bailey, M., Fenlon, J., Fermor, T., Lilley, A., Lynch, J., McCormack, P., McQuilken, M., Purdy, K. & Rainey, P. (1993). Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (Beta vulgaris). *Plant and Soil*, 150(2), pp. 177-191.

Trotel-Aziz, P., Couderchet, M., Biagianti, S. & Aziz, A. (2008). Characterization of new bacterial biocontrol agents Acinetobacter, Bacillus, Pantoea and Pseudomonas spp. mediating grapevine resistance against Botrytis cinerea. *Environmental and Experimental Botany*, 64(1), pp. 21-32.

Tukey Jr, H. (1970). The leaching of substances from plants. *Annual review of plant physiology*, 21(1), pp. 305-324.

Vaas, L.A., Sikorski, J., Hofner, B., Fiebig, A., Buddruhs, N., Klenk, H.-P. & Göker, M. (2013). opm: an R package for analysing OmniLog® phenotype microarray data. *Bioinformatics*, 29(14), pp. 1823-1824.

van der Horst, M.A. & Hellingwerf, K.J. (2004). Photoreceptor proteins,"star actors of modern times": a review of the functional dynamics in the structure of representative members of six different photoreceptor families. *Accounts of chemical research*, 37(1), pp. 13-20.

van der Horst, M.A., Key, J. & Hellingwerf, K.J. (2007). Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. *Trends in microbiology*, 15(12), pp. 554-562.

Van Ieperen, W. & Trouwborst, G. The application of LEDs as assimilation light source in greenhouse horticulture: a simulation study. In: *Proceedings of International Symposium on High Technology for Greenhouse System Management: Greensys2007 801*2007, pp. 1407-1414.

Varnier, A.L., Sanchez, L., Vatsa, P., Boudesocque, L., GARCIA-BRUGGER, A., Rabenoelina, F., Sorokin, A., RENAULT, J.H., Kauffmann, S. & Pugin, A. (2009). Bacterial rhamnolipids are novel MAMPs conferring resistance to Botrytis cinerea in grapevine. *Plant, Cell & Environment*, 32(2), pp. 178-193.

Vartoukian, S.R., Palmer, R.M. & Wade, W.G. (2010). Strategies for culture of 'unculturable'bacteria. *FEMS microbiology letters*, 309(1), pp. 1-7.

Vokou, D., Vareli, K., Zarali, E., Karamanoli, K., Constantinidou, H.-I.A., Monokrousos, N., Halley, J.M. & Sainis, I. (2012). Exploring biodiversity in the bacterial community of the Mediterranean phyllosphere and its relationship with airborne bacteria. *Microbial ecology*, 64(3), pp. 714-724.

Vorholt, J.A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, 10(12), pp. 828-840.

Vox, G., Teitel, M., Pardossi, A., Minuto, A., Tinivella, F. & Schettini, E. (2010). Sustainable greenhouse systems. *Sustainable agriculture: technology, planning and management. Nova Science Publishers, Inc., New York, NY, USA*, pp. 1-79.

Warren, R. (1972). The effect of pollen on the fungal leaf microflora of Beta vulgaris L. and on infection of leaves by Phoma betae. *Netherlands Journal of Plant Pathology*, 78(3), pp. 89-98.

Werner, G.D., Strassmann, J.E., Ivens, A.B., Engelmoer, D.J., Verbruggen, E., Queller, D.C., Noë, R., Johnson, N.C., Hammerstein, P. & Kiers, E.T. (2014). Evolution of microbial markets. *Proceedings of the National Academy of Sciences*, 111(4), pp. 1237-1244.

Whipps, J., Hand, P., Pink, D. & Bending, G.D. (2008). Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of applied microbiology*, 105(6), pp. 1744-1755.
Whitelam, G.C. & Halliday, K.J. (2008). *Annual Plant Reviews, Light and Plant Development*30): John Wiley & Sons.
Williamson, B., Tudzynski, B., Tudzynski, P. & van Kan, J.A. (2007). Botrytis cinerea: the cause of grey mould disease. *Molecular plant pathology*, 8(5), pp. 561-580.
Wink, M. (2010). Functions and biotechnology of plant secondary metabolites. Annual Plant Reviews 39. Blackwell Publishing Ltda, USA.
Wong, T.G. & Ackerly, D.D. (2005). Optimal reproductive allocation in annuals and an informational constraint on plasticity. *New Phytologist*, 166(1), pp. 159-172.
Wu, L., McGrane, R.S. & Beattie, G.A. (2013). Light regulation of swarming motility in Pseudomonas syringae integrates signaling pathways mediated by a bacteriophytochrome and a LOV protein. *MBio*, 4(3), pp. e00334-13.
Yadav, R., Halley, J., Karamanoli, K., Constantinidou, H.-I. & Vokou, D. (2004). Bacterial populations on the leaves of Mediterranean plants: quantitative features and testing of distribution models. *Environmental and Experimental Botany*, 52(1), pp. 63-77.
Yadav, R., Karamanoli, K. & Vokou, D. (2005). Bacterial colonization of the phyllosphere of Mediterranean perennial species as influenced by leaf structural and chemical features. *Microbial ecology*, 50(2), pp. 185-196.
Yan, L., Jing, T., Yujun, Y., Bin, L., Hui, L. & Chun, L. (2011). Biocontrol efficiency of Bacillus subtilis SL-13 and characterization of an antifungal chitinase. *Chinese Journal of Chemical Engineering*, 19(1), pp. 128-134.
Yang, C.-H., Crowley, D.E., Borneman, J. & Keen, N.T. (2001). Microbial phyllosphere populations are more complex than previously realized. *Proceedings of the National Academy of Sciences*, 98(7), pp. 3889-3894.
Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F.O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzéby, J., Amann, R. & Rosselló-Móra, R. (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology*, 12(9), pp. 635-645.
Yashiro, E., Spear, R. & McManus, P. (2011). Culture-dependent and culture-independent assessment of bacteria in the apple phyllosphere. *Journal of applied microbiology*, 110(5), pp. 1284-1296.
Yeh, N. & Chung, J.-P. (2009). High-brightness LEDs—Energy efficient lighting sources and their potential in indoor plant cultivation. *Renewable and Sustainable Energy Reviews*, 13(8), pp. 2175-2180.
Yin, R., Dai, T., Avci, P., Jorge, A.E.S., de Melo, W.C., Vecchio, D., Huang, Y.-Y., Gupta, A. & Hamblin, M.R. (2013). Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. *Current opinion in pharmacology*, 13(5), pp. 731-762.
Youssef, N.H., Duncan, K.E., Nagle, D.P., Savage, K.N., Knapp, R.M. & McInerney, M.J. (2004). Comparison of methods to detect biosurfactant

production by diverse microorganisms. *Journal of Microbiological Methods*, 56(3), pp. 339-347.

Zukauskas, A., Bliznikas, Z., Breivė, K., Novičkovas, A., Samuolienė, G., Urbonavičiūtė, A., Brazaitytė, A., Jankauskienė, J. & Duchovskis, P. Effect of supplementary pre-harvest LED lighting on the antioxidant properties of lettuce cultivars. In: *Proceedings of VI International Symposium on Light in Horticulture 907*2009, pp. 87-90.

Acknowledgements

Although acknowledgement is the last part of the PhD thesis, still I believe it is the most vital part of it. I am pleased to reflect on all my memories and feel thankful to all people who has played important roles in this challenging journey.

I would like to thank my main supervisor, *Beatrix Alsanius* for giving me the opportunity to do my PhD under your supervision and inviting me to daily challenges. I have learned a lot from you, not only about science but also about life. You taught me how to improve my skills and I have become a better professional in this field under your supervision. Thank you for all of the enjoyable discussions during our walks. I have learned that life can be really tough sometimes, but that I should not give up and that I have to fight for my goals. Without your distinctive supervision, my project would not have been materialized.

I wish to express my thanks to my co-supervisors, *Sammar Khalil*, *Walter Wohanka*, *Sofia Windstam* and *Håkan Asp* for all your support and great input to the projects and manuscripts.

Thanks to EU Interreg North Sea Program IVB; project "GreenGrowing: Towards a smaller carbon footprint in the horticultural greenhouse industry", for funding this project.

I would like to sincerely express my warm regards and deep sense of appreciation to *Linda Tufvesson* for all your endless support.

Many, many thanks to *Lars Mogren* for always being ready to listen to me, give advice and encourage me even at difficult times. Thanks for your positive

energy and happiness, which you always brought not only to me but also to our working environment. You are a bundle of greenish positive energy. Stay green and happy!

Special thanks to *Anna-Karin Rosberg*. For your willingness to help whenever asked for and giving me wise consult in different occasions. Simply, I enjoyed sharing office with you.

My deep acknowledge to *Christer Olsson*, for helping me in the lab patiently and keeping my heart warm by your smile. Stay blessed.

Many thanks to *Karl-Johan Bergstrand* for all scientific discussions and great times during our business trips and conferences abroad.

Rahel, I was lucky enough to have your free hand during frustrating phase of my night experiment, besides you were an amazing office mate. Thanks a lot!

I was very grateful to have to you both, *Emina Mulaosmanovic* and *Maria Sousa*, beside me during the last year of my PhD. It has been a great time. Thanks a lot for caring about me and being there for me even 'after work' and keep asking if I needed any help. During these crucial stage of my PhD education you were always there to cheer up when things weren't working well. I wish you all the best things in life!

A big thank to *Lea Vaas*! Meeting you in Florence, September 2015, opened new doors in my PhD project. We wouldn't have reached to these valuable results without you.

I would also like to thank:
Maria Karlsson for support during literature exam, *Julia Lindén* and *Maria Grudén* for making the work environment more enjoyable. *Malin Hultberg* for help with the biosurfactant test. *Andrea Kosiba Held*, concerning scientific discussions during her short stay. *Helena Karlen*, *Peter Andersson* and *Elisabeth Marling* for all the fun around the coffee table!

Helena Person Hovmalm and *Helene Larsson Jönsson* for support concerning PhD education.

Agnetha Karlberg and *Gunilla Andersson* for administrative support.

Christina Johansson, for help with the accommodation in Alnarp.

To the Microhort research school participants, for having great time during the courses and nice chances to meet new PhD students.

I am thankful to *Justine*, *Christine*, *Faraz*, *Shaheed*, *Lucie*, *Galen* and *Theoharis* for nice time and friendship.

My friends in Sweden, *Mike* (you always encouraged me and believed in me, you never left me alone), *Santosh* (you were always there for me to listen) and *Suzan* (you were always there to hear my woes and let me lighten my heart), thanks for our great friendship, for making lots of good memories and for being by my side in ups and downs. Stay friends☺!

Shahram, my wise uncle, you were next to me from the first day that I came to Alnarp. After my parents, you are the one who always listened to me patiently, gave me great advice, and kept me going.

Hengameh and *Shahriyar*, I will never forget that you kindly hosted me during the first few months of my PhD study as a member of your family. I am really thankful for all of your kindness!

My family in Sweden, My godmother *Mahvash*, I am so lucky and thankful to have you close by. Thanks for treating me like (Pega and Pani). I will always remember your song: Taghat biyar.

پدر ام، پگاه، پانی Many thanks for endless support and boundless fun!

Sara, I am so happy to have my old-gold friend not that far away. Thanks for hosting me during Norouz and that you keep inviting me to Stockholm.

My friends back home, *Shima*, *Khatoon*, *Sanaz*. Thanks for giving me hope all the time and make me smile.

Finally:

My **wonderful parents**, *Hossein* and *Shohreh*, my deepest gratitude is for you, who encouraged me to continue my education, supported me financially during my PhD study and pushed me forward in all hard situations. Without your support, I could not reach to this point. I hope I could make you a little bit more proud of me by my achievements. *Sahar*, my dear sister, even far away, I always felt you standing by my side, talking with me on phone, trying to give me strength. Thanks for taking care of our parents during my absence.