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Diplomarbeit

Trichoderma Photobiology

ausgeführt am

Institut für Verfahrenstechnik, Umwelttechnik und

Technische Biowissenschaften

unter der Anleitung von

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Zusammenfassung

Sichtbares Licht ist ein Bestandteil des elektromagnetischen Spektrums, das nicht nur auf autotrophe Organismen (Photosynthese) einen positiven Einfluss hat, sondern sich auch auf die Regulation von heterotrophen auswirkt. Dies ist in Prozessen wie den Circadian Rhythmen, der Ontogenese, der Vermehrung oder in deren Ausbreitung in der Umwelt etc. zu beobachten. Bei den Pilzen hängen viele biologische Faktoren vom Einfluss des Lichtspektrums ab. Die Hauptaufgabe in meiner Arbeit war es zu bestimmen, ob die Pilze der verbreiteten Gattung *Trichoderma* auf Blaulicht reagieren.

Im Zuge dieser Arbeit entwickelten wir eine effiziente Methode zur Beurteilung des Blaulichteffekts auf das Wachstum und die Konidien Ausbildung der *Trichoderma*. Für diese Aufgabe testeten wir 100 wildtypen Pilze, von denen 52 Stämme zur Gattung *Trichoderma* gehörten. Dieser Effekt wurde mit *Asperigillus fumigatus* und *Aspergillus nidulans* und mit dem Resultat eines anderen mykoparasitischen Pilzes *Escovopsis weberi* verglichen. Unsere ursprüngliche Hypothese besagte, dass die Reaktion auf Illumination mit der Evolution der *Trichoderma* zusammenhängt. So wurden lichtempfindliche, lichtinhibiterte und lichtneutrale Stämme in Gattungen und Taxa erläutert.

Die Ergebnisse des Experiments zeigten, dass das Geschlecht *Trichoderma* prinzipiell nicht lichtempfindlich ist. Nichts desto weniger konnten wir einige *Trichoderma* Stämme ausfindig machen, die entweder stark lichtstimuliert oder stark lichtinhibitent waren. Um dies zu erklären, erforschten wir ebenso die Transkriptionale Antwort von Gene, die bei der Photoregulation (*phr1*, *nox1* und *lae1*) beteiligt waren und unterzogen sie einigen Tests.

Die in dieser Arbeit erhobenen Daten können später wieder als Grundlage zu weiteren Experimenten, die die Photostimulation des Geschlechts *Trichoderma* beinhalten, herangezogen werden.

Abstract

Visible light is a part of the electromagnetic spectrum that may positively influence not only autotrophic organisms (photosynthesis) but it also effects heterotrophs regulating such processes as circadian rhythms, ontogenesis, reproduction and dispersion in the environment etc. In fungi many biological processes are related to the influence of the light spectrum. The main goal of this work was to determine if fungi belonging to the common mycotrophic genus *Trichoderma* respond to blue-light. In this study we developed a semi-high throughput method for the assessment of blue-light effect on growth and conidiation of *Trichoderma*. For this purpose we have tested 100 wild-type strains belonging to 52 species from the genus *Trichoderma*. The effect was compared to *Asperigillus fumigatus* and *A. nidulans* and to the response of another mycoparasitic fungus *Escovopsis weberi*. Our initial hypothesis was that response to illumination is correlated to the evolution of the *Trichoderma*. Thus, photosensitive, photoinhibited and photoneutral strains in species and infrageneric clades were elucidated.

Results of the experiments showed that in general the genus *Trichoderma* is not photosensitive. However we were able to detect some of *Trichoderma* strains that are either strongly photostimulated or photoinhibited. To explain this we also investigated the transcriptional response for the genes for which the involvement in the photo regulation, beside blue-light receptors *brl-1* and *brl-2*, was recently reported (*phr1*, *nox1* and *lae1*) was tested.

The data obtained in this work can be used subsequently as a basis for further experiments related to the photostimulation of *Trichoderma* genus.

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List of abbreviations

UV – ultra violet

IR – infra red

ROS – reactive oxygen species

spp. – Species pluralis

nm – nanometers magnitude of wavelength

cDNA – complementary DNA

gDNA – genomic DNA

RT-PCR – reverse transcription polymerase chain reaction

T_m – primer melting temperature

1. Introduction

1.1. Photobiology: light sensing and response of living organisms

The light wave could be characterized by different points such as intensity, duration, polarization and spectral composition. All these parameters are important for living organisms and influence them in different ways (Mukherjee et al. 2013).

The electromagnetic spectrum could be divided in several types of waves (Fig.1.2.1). Radiation of longer wavelength, such as infrared light, is carrying less energy. On the other hand ultraviolet waves have shorter wavelength and containing more energy. This proportion between wavelength and carried energy is true for all electromagnetic spectrum ("Electromagnetic Spectrum" 2014).

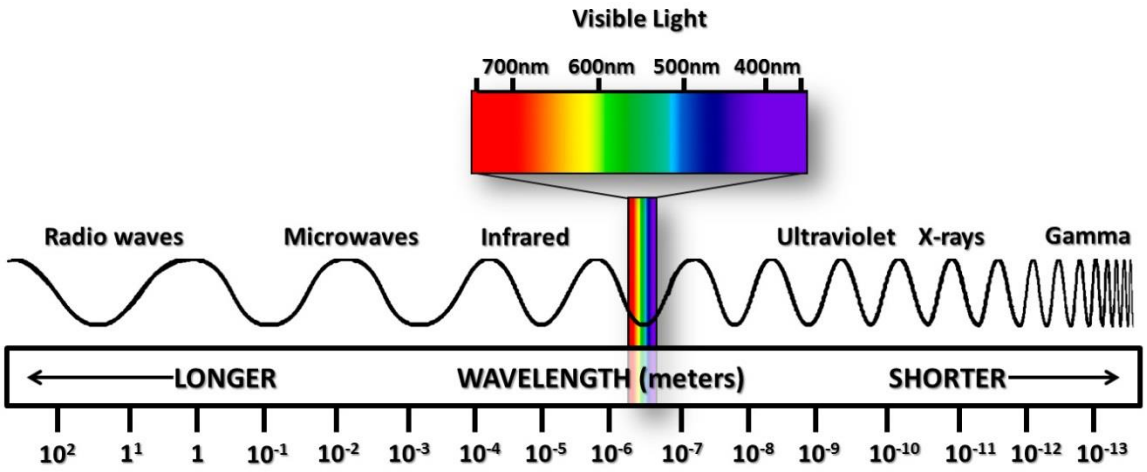

Figure 1.1.1 "Electromagnetic spectrum" (CSI)

Almost all living organisms are exposed to different electromagnetic radiation during the life cycle. The electromagnetic radiation between UV and IR (visible light) could initiate several photochemical reactions in living organisms such as circadian rhythms, photo morphogenesis, phototropism, synthesis of pigments etc. However not all of the effects are positive. UV radiation and blue light can cause mutations and can initiate uncontrolled free radical reactions that involving

reactive oxygen species (ROS) (Mukherjee et al. 2013). However, the sun energy is used by the plants to produce sugars molecules from inorganic compounds such as carbon dioxide and water. These molecules are then used directly by the plants or indirectly by animals, bacteria and fungi.

Light is essential for every type of plants. During the photosynthesis process the energy of light could be converted to sugar molecules. But it is not the only way how electromagnetic spectrum impact plants. Plants are using the light not just as energy source but also as a valuable signal of the environment and respond to its intensity, wavelength, and direction. Plants detect light quality by at least three families of photo-receptors: phytochromes, cryptochromes and phototropins. Phytochrome absorbance peaks are in the red (600 to 700 nm) and far red (700 to 800nm) light, and to a smaller interval in blue (400 to 500 nm) light (Runkle and Heins 2001). Plants perform a wide range of varieties of physiological responses using the received information from photoreceptors. The development and the physiology of the plant are strongly attached to the available light spectrum of the environment. Blue light spectrum involved in such processes as phototropism, photo-morphogenesis, stomatal opening, and leaf photosynthetic functioning. It was measured that the absorption of blue and red light by the plant may rich 90% level, that underlines again the importance of light, as an essential factor influencing on plants. In contrast, green light has been reported to be negative on physiological and developmental incomes (Muneer et al. 2014).

Regulatory biochemicals of plants are effected by the available light spectrum of the environment. One of such examples could be auxin, a plant hormone that is responsible for the plant's stem grows. If there is sufficiency of blue light, in nature environment this period usually refers to autumn and winter time, than chryptochrome photo receptor inhibits the activity of the stem growth hormone. Another example tells us that plants use the quantity of blue light to determine how far to open their stomas. The more blue light, the wider they open their stomas, so accelerating their

metabolism. High levels of blue light will therefore promote increased metabolism, and by extension accelerate plant growth and development ("The Influence of Colours on Plants" 2014).

Studies also showed that life cycle of marine plants could be influenced by waves with different length. A pulse of blue light causes a rapid and significant increase in the rate of photosynthesis in the brown algae. However, there is no effect in green and red algae that were tested. The greatest response inside of the group of brown algae was observed in species from the littoral zone. Brown algae with a thin or filamentous morphology exhibited a higher degree of blue light stimulation than species with thicker thalli (Forster and Dring 1994). It is shown that the blue light could be a trigger for carbon dioxide metabolism in marine plants (Sorek and Levy 2012).

In *Drosophila melanogaster*, one of the best studied eukaryotic model organisms, the blue light was used to synchronize endogenous time with the daily cycles (Gentile et al. 2013) which in turn controls the right functioning of biorhythms such as duration of the "mating song", oviposition, embrio and imago development (Yao and Shafer 2014).

One of the best studied heterotrophic organisms in regards to the light sensing and response are certainly fungi. The first fungus in which the effect of light was analyzed was probably *Phycomyces*. These fungi are known for their strong phototropism response and helical growth of the sporangium. The most studied species is *Phycomyces blakesleeanus*. The pioneer studies on this species were done by Max Delbrück starting from 1950-th (Bergman et al. 1969). Delbrück was trying to study signal transductions pathways associated with light. His work represented a competent basis for a development of the fungal photobiology and it was related to the capacity of this fungus to "see" the light.

Another excellent example of the phototropism in fungi is *Pilobolus* genus. The sporangiophore (asexual fruiting structure) develops on a stem and has an ability to orientate itself to the light source. After maturing period the sporangium could be launched using the pressure within the subsporangial

vesicle to a distance of 2 meters with extraordinary speed (Yafetto et al. 2008); (Kubo and Mihara 1989).

Neurospora crassa is one of the fungi in which influence of light on the circadian cycle was studied in detail (Jay C Dunlap and Loros 2004). The first blue light photoreceptor was found in *Neurospora crassa* (Froehlich et al. 2002) and for today it is a tractable model system for understanding the molecular bases of circadian rhythms in eukaryotes (Dunlap et al. 2007). Light affects a variety of physiological processes in *Neurospora,* including entrainment and resetting of the circadian clock, biosynthesis of the photo-protective pigments, induction of asexual conidiospores, development of sexual structures, and the direction of ascospore dispersal (Chen, Dunlap, and Loros 2010). After decades of studies, all known light responses in *Neurospora* are restricted to near UV/blue-light, suggesting the presence of blue-light photoreceptors. Two proteins called white-collar 1 and white-collar 2 (WC-1 and WC-2) are the main components of blue light sensing system. Both proteins are showing characteristic features of transcriptional factors such as zinc finger and putative transcriptional activation domains. Additionally, LOV (light, oxygen or voltage) domain, which contains chromophore, is presented just in WC-1. On the other hand PAS (PER-ARNT-SIM) domains, which are important for the formation of White-Collar Complex (WCC) are introduced in both proteins (Schwerdtfeger and Linden 2000; Schwerdtfeger and Linden 2003). WCC complex is presented in light and dark conditions of the environment. Under the blue light photostimulation WC-1 is phosphorylated. It is possible to detect that the phosphorylation level increases after 15 min of the illumination which is followed by degradation of WC-1. Proportions of WC-1 and WC-2 are staying constant that leads to assume that WC-1 is synthesized continuously while WC-2 stays unmodified (Chen, Dunlap, and Loros 2010). The WCC complex is strictly situated in the nucleus and transducts the light signal to the blue light regulated promotors (Schwerdtfeger and Linden 2003).

Another protein that is important for the blue light response and has been identified in *N. crassa* is VIVID. This is a small protein (21kDa) and is associated with a flavin-type chromophore (Chen and Loros 2009). VIVID has important roles within the *Neurospora* circadian system. Without VIVID the organism is more sensitive to light, resulting in the rapid breakdown of circadian organization in continuous illumination. By influencing clock resetting at both dawn and dusk, VIVID affects the circadian clock. Finally, VIVID plays a role in maintaining the correct timing of clock-controlled output pathways at different temperatures (Hunt et al. 2010). VIVID contains a LOV domain and it was found only in cytoplasm of mycelia cell. Its level increases after the photostimulation (Schwerdtfeger and Linden 2003). As WCC is located in nucleus it shows that direct interaction with VIVID is not possible but VIVID functions downstream of the WCC to regulate negatively the light responses initiated by the WCC (Chen et al. 2010).

Processes that are regulating the *Neurospora* circadian system are shown on the picture bellow (Fig. 1.1.2). The WC-1 and WC-2 proteins form a White Collar Complex (WCC) that activates *frq* gene expression and also *clock-controlled gene* (*ccg*, output) and *vvd* expression in the dark. The WCC also regulates light-induced transcription of *frq*, *ccg* genes, *vvd*, and *wc-1* (gold arrows).The expression of VIVID is strongly induced by the light. In the same time VIVID is a photoreceptor that regulates light adaptation responses by turning down the WCC activity for a short periods of time. In the dark conditions *frq* genes are translated to FRQ proteins that bind to dimers and have two main functions. First one is to feed back in to the nucleus and block the activity of WCC in *frq* gene transcription. The second function is to promote the synthesis of WC-1 and WC-2 (it will lead to making more WCC, which is held to be inactive by FRQ). Phosphorylation of FRQ (by kinases) triggers it turnover. The phosphorylation-mediated turnover is a major determinant of period length in the clock. When FRQ is degraded in the proteasome, the pool of WCC is released to reinitiate the cycle (Borkovich et al. 2004).

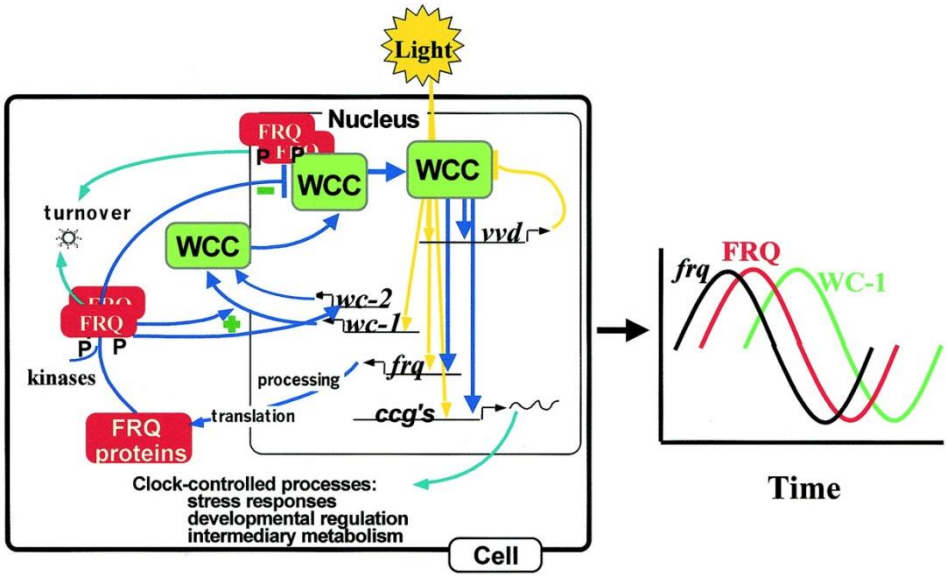

Figure 1.1.2. Circadian system in *Neurospora crassa* cells (Borkovich et al. 2004).

It is possible to summarize that VIVID enables the fungus to sense changes in light conditions and adapt to them (Gin et al. 2014).

1.2. Case studies of photostimulation in *Trichoderma*

The light responses of *Trichoderma* genus are similar in many ways to those of *N. crassa*. *Blr-1* and *blr-2* in *Trichoderma* are orthologous genes to *wc-1* and *wc-2*, respectively. The BLR-1 protein has all the characteristics of a blue-light photoreceptor, whereas the structure of the deduced BLR-2 protein suggests that it interacts with BLR-1 through PAS domains to form a complex. *Blr-1* and *blr-2* are essential for blue-light induced expression of some genes (Casas-Flores et al. 2004). One of such genes in *T. harzianum* and *T. atroviride* was recently identified *phr1*, which predicted product is the DNA repair enzyme photolyase (G M Berrocal-Tito et al. 2000). DNA photolyases are able to use the sun light for the repair of DNA. The process of repair of DNA in organisms using photolyases and sun light is called photoreactivation. Photolyases have two chromophores in the structure, a

catalytic chromophore flavin and a light harvesting one which is either methyl tetrahydrofolate (MTHF) or another flavin (Gloria M. Berrocal-Tito et al. 2007). Photolyases bind to damaged DNA, and during the activation of flavin, they generate a dimer radical that breaks down, restoring the damaged pair of located near pyrimidins (Gloria M. Berrocal-Tito et al. 2007). Products of *phr1* gene are accumulated in the vegetative mycelia and conidiophores (G. Berrocal-Tito et al. 1999). It was suggested that photolyases are protecting *Trichoderma*, which grows in a soil as a mycoparasite or saprophyte, against the harmful UV damage (G M Berrocal-Tito et al. 2000).

Another example of a gene which expression is regulated by the blue light is *nox1*. This gene is encoding NADPH oxidase enzyme that is involved in the production of reactive oxygen species (ROS) in animals and plants as an earlier defense response on mechanical injuries and protection against bacteria and fungi. Three different *Nox* subfamilies have been found in fungi: *NoxA* (*Aspergillus nidulans*; possess domains for catalytic core), *NoxB* (has an additional N-terminal extension) and *NoxC* (longer N-terminal region with a putative calcium binding EF-hand motif). At the same time in various fungi reduced levels of NOX enzymes may effect on sterility, decrease asexual development, block the production of fruiting bodies and reduce the hypha growth (Hernandez-Onate et al. 2012). *Trichoderma* includes species which are applied as biological control agents due to their ability to antagonize other fungi what is directly related to the production of NOX enzymes (Montero-Barrientos et al. 2011; Hernandez-Onate et al. 2012).

Furthermore, *lae1* gene has a dynamic role in the morphological and chemical development of fungi (Karimi-Aghcheh et al. 2013). In Ascomycota the protein methyltransferase *LaeA* is a global regulator that affects the expression of secondary metabolite gene clusters and controls sexual and asexual development. Several experiments were made with *T. atroviride* to see the influence of the deletion and overexpression of *lae1* gene on nutritional and eco-physiological characteristics of the strain. *T. atroviride* was chosen as a model for the experiment as the most studied agent of biological

control of phytopatogenic fungi. In the presence of light strains with *Δlae1* are showing 50% decrease of conidiation level and in contrast strains with *lae1* overexpressed strains (*OElae1*) are showing 30-50% increase of the conidiation level. In the darkness *Δlae1* strains lost sporulation also even after mechanical injuries. Karimi-Aghcheh et al. (2013) demonstrated increased sensitivity to oxidative stress that lead to a loss of mycoparasitic behavior. On the other hand *OElae1* strains showing the same sporulation level in the darkness as the parent strain and enhanced mycoparasitic abilities (Aghcheh et al. 2013). Experimental data demonstrate that the putative protein methyltransferase *LAE1* is essential for cellulase gene expression in *T. reesei* (Seiboth et al. 2012).

In earlier researches it was noticed that photostimulation or photoinhibition of growth and conidiation of *Trichoderma* genus is dependent on the available carbon sources. Species of *Trichoderma* are soil saprophites and facultative mycoparasites, which can also be found in the rhizosphere or become plant symbionts (Friedl et al. 2008). They are usually found on the surface of decaying plants rests and litter layer. Soil fungi are using aerial conidiation as the most efficient way for reproduction and dispersal (Mukherjee et al. 2013). In this case the problem is caused by the fact that the availability of one or another single nutrient compound alone cannot guarantee that the hyphae will reach the most successful area for spore dispersion. In this case the sense of the light can be an advantage for the fungi. It will signal the presence of aerial environment to the fungi and will lead to the production and sporulation in the shortest possible time (Friedl et al. 2008). All that underlines the importance of light for conidiation and growth of the fungus.

T. reesei is known as one of the most efficient plant cell wall degrader. Expression of the enzymes for this process is regulated not just by nutritional signals but also by other environmental signals such as light (Tisch and Schmoll 2013). Last experiments are showing that strains with the lack of the photoreceptors (BLR1, BLR2 and ENVOY1 (ENV1; equivalent of PAS/LOV domain)) are represented by a greater number of genes showing significantly different transcript levels in light

and dark conditions. Glycoside hydrolases (including cellulose and hemicellulose), main enzymes involved in the degradation of the plant cell walls, can be a subject to light dependent regulation (Tisch and Schmoll 2013).

1.3. Biology and applications of the genus *Trichoderma*:

Trichoderma genus is one of the most frequent fungi detected all over the world in variable environments. First description of this genus was made by Christian Hendrik Persoon in the eighteenth century (Persoon, 1794). Fungi of this genus have been isolated from numerous of natural and artificial substrates that confirm their high opportunistic potential and adaptability to various ecological conditions. Among hundreds of fungal genera, *Trichoderma* has a huge impact on human's welfare. Some *Trichoderma* species are high producers of enzymes that are used for a reduction of plant biomass to simple sugars that can be used for biofuel production and other biorefinery processes (Druzhinina and Kubicek 2013). The best example could be *Trichoderma reesei* that is domesticated and commercially used. Mutants of first isolated QM6a strain are used nowadays for production of polysaccharide hydrolytic enzymes and heterologous proteins (Saloheimo and Pakula 2012; Kumar, Singh, and Singh 2008). *Trichoderma* is also known for another feature to parasitize on other fungi. This ability is widely used to control phytopatogenic fungi (Kubicek and Harman 1998). It is also used as a biocontrol agent of nematodes. The mycoparasitic traits of *Trichoderma* can also cause negative effects. Some of the strains like *T. agressivum*, *T. pleuroticola*, *T. pleurotum* and *T. mienum* are antagonistic to the commercial mushrooms *Agaricus* and *Pleurotus*. Some of the *Trichoderma* strains isolated from tropical and subtropical ecosystems have been described as symptomless associates of plants or endophytes (Druzhinina and Kubicek 2013). However, it was also shown that some strains from section *Longibrachiatum* that may be important in medical context as they could

be opportunistic pathogens of immunocompromised mammals including humans (Druzhinina et al. 2008).

Trichoderma genus as a representative of ascomycete fungi is haploid during the vegetative stage of the life cycle and has a heterothallic mode of sexual reproduction. It means that mating is only possible between individuals with different mating types genes *(mat1-1* and *mat1-2* respectively). Large number of *Trichoderma* species till today have no confirmations about teleomorph stage of the development and they are considered to be clonal. *T. hamatum, T. tomentosum, T. cerinum T. pleuroticola, T. spirale, T. aggressivum, T. gamsii* are representing that tendency (Druzhinina and Kubicek 2013).

As it was mentioned before recognition of *Trichoderma* species and work on their taxonomy started more than seventy years ago after the II World War. Today the genus *Trichoderma* is exceptionally well documented by DNA barcoding and molecular evolutionary analyses with use of the universal for all fungi DNA barcode markers ITS1 and 2 of the rDNA gene cluster, the fourth and fifth introns of translation elongation factor 1-alpha (*tef1*), a partial exon of endochitinase *chi18-5* (formerly *ech42*), partial intron containing sequences of calmodulin (*cal1*) and actin (*act*) genes, the coding fragment of the RNA polymerase subunit B II gene (*rpb2*) and some other markers (Druzhinina and Kubicek 2013). Most of the researches concerning enzyme production (food, feed, biofuel and heterologous protein production industry), biofungicides, biofertilizers, improved seed germination, source of transgenes, herbicides, production of drugs of clinical significance, human patogens, ecological studies and others (Atanasova et al. 2013) could be not possible without the availability of DNA barcoding (Druzhinina and Kubicek 2013).

Previously, it was assumed that *Trichoderma* is a soil fungus. This assumption was supported by the fact that the majority of isolations were made from soil samples world-wide. Qualitative analysis of the diversity of strains in such samples shows the dominance by the same 15-20 highly

opportunistic species (Irina S. Druzhinina and Kubicek 2013). It shows that these species obtained the ability of saprotrophic growth in soil environment but this is not supporting the idea about *Trichoderma* as soil fungi (Irina S. Druzhinina and Kubicek 2013). Despite the fact that *Trichoderma* species are showing associations with higher plants, basidiomycetes, invertebrates, soil nematodes and mammals most of the represented taxa have been isolated from dead wood rests and fruiting bodies of other fungi that underlines another ecological niches of this genus. It seems that that the role of *Trichoderma* in microbial community is related to the ability to pray on other fungi and (or) to inhibit their growth by production of antifungal metabolites (Druzhinina and Kubicek 2013).

Nowadays 12 genomes of *Trichoderma* species (*T. parareesei, T. asperellum, T. harzianum, T. reesei, T. atroviride, T. longibrachiatum, T. virens and T. afroharzianum*) are sequenced and available in public and private databases. Detailed genome analyses can make a great impact and open new possibilities in industry, agriculture and ecological branches of science.

1.4. Importance of the *Trichoderma* genus for different fields of study

The genus *Trichoderma* has a great effect on different branches of science and on the human's life directly. Members of genus *Trichoderma* are well adapted to different environmental conditions and presented in a wide range of climatic zones. Success in different ecological niches is caused by metabolic diversity, high reproductive capacity and competitive capabilities of *Trichoderma* genus (Gupta 2014).

In the Second World War the USA army was extremely concerned at the rate at which their uniforms and cellulosic materials decayed in the tropics. Interestingly that the most powerful cellulolytic mutants from that time have been derived from *Trichoderma reesei* QM6a. *Trichoderma reesei* QM6a strain was isolated from the rotting cartridge belt used in the jungles of New Guinea.

As a result of these finding new laboratories were founded on the territory of USA to investigate the mechanisms of cellulose degradation (Coughlan and Hazlewood 1993).

Strains of the *Trichoderma* genus, their mutants and biotechnological products are irreplaceable in many branches of the industry. *Trichoderma* spp. are superior producers of many extracellular enzymes that can efficiently degrade complex polysaccharides (Kubicek and Harman 1998). This provides a lot of great opportunities for the food, feed and textile industries. For example, cellulases from these fungi are used in "biostoning" of denim fabrics to get softer product. Enzymes are also used in poultry feed to increase the digestibility of hemicelluloses from barley or other crops (Kubicek and Harman 1998). Furthermore, *Trichoderma* spp. are used in agriculture as biocontrol agents against plant pathogenic fungi. Additionally, the ability of these fungi to stimulate plant growth and development, including, their ability to increase the development of more robust roots has been known. The mechanisms for these abilities are only just now becoming known and have a big future (Kubicek and Harman 1998).

2. Aims of the study

The aim of the thesis was to investigate the response of *Trichoderma* genus to illumination on the broad generic level. In order to pursue this aim the following objectives have been formulated:

i) the semi-high throughput method for the assessment of fungal response to illumination has to be developed;

ii) based on this method the analysis of photoresponse *Trichoderma* fungi covering all major species and infrageneric phylogenetic clades has to be performed. The comparison with some other filamentous Ascomycota has to be made.

We were interested to explore if *Trichoderma* genus has a photosensitive, photoinhibited or photoneutral tendency. We wanted to see if there are any strains that violate the pattern. Moreover we wanted to investigate the correlation between the influence of the blue light spectrum and the evolution of *Trichoderma* genus.

iii) the results of the experiments have to be investigated on the molecular level through the expression analysis of the genes putatively involved in photoregulation in some model *Trichoderma* strains.

3. Materials and methods

3.1. Strains used in this study

In this study 130 fungal strains were used. Among them 98 and 25 were wild-type and mutant *Trichoderma* strains respectively; we also tested *Aspergillus fumigatus* (2 strains) and *A. nidulans* (3 strains) and *Escovopsis weberi* (1 strain). Most of the mutants were included to study the impact of the genes involved in light response of *Trichoderma* and *Aspergillus*. The details about the studied strains such as database numbers, species name and origin of the sample are presented in the Tables 3.1.1.a and 3.1.1.b. Most of the samples were isolated from plants, soil and decaying wood debris. Nevertheless, few of them were obtained from old human's settlements or tombs, ant nests of *Atta cephalotes* and *Camponotus cylindricus* as well as from marine sponge *Psammocinia* sp. This additionally underlines the prevalence of *Trichoderma* genus habitats.

The isolates are stored at -80ºC in 50% glycerol in the collection of Industrial Microorganisms of Vienna University of Technology (TUCIM). For convenience, TUCIM numbers (C.P.K.) are used for the strains in the thesis. All strains were precultivated on malt extract plates (MEX plates: 20 g/l agar-agar, 30 g/l malt extract) and subcultured every two weeks. The strains were incubated at 25 ºC and 12 hours of cyclic light.

Special source of light was used for experiments. Two "FLUORA T8" lamps produced by Osram were installed in the incubator. An intensity of the light produced for this experiment equals to 2600 lux ("FLUORA T8 | Leuchtstofflampen T8 Spezialversionen | OSRAM" 2014). Light spectrum of the lamp shows peak around 450 nm that refers to the blue light wave length (Fig 3.1.1). A diurnal light cycle established for experiments starts with 12 hours of darkness after which follows 12 hours of the photostimulation.

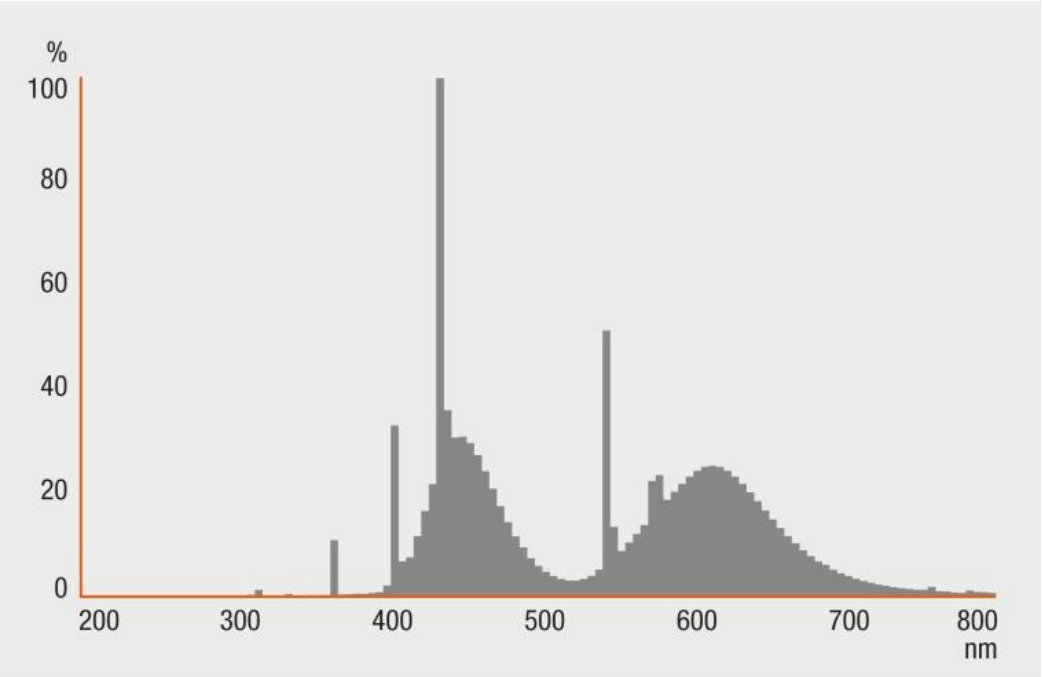

Figure 3.1.1 “Fluora” lamp light spectrum (x-axis shows the distribution of radiation, y-axis shows the wavelength).

Table 3.1.1.a. Wild-type strains of *Trichoderma* and other fungi used in this study.

№	C.P.K.	Other collection codes	Species	Country	Origin
1	717	ATCC MYA-4777	*T. parareesei*	Mexico	soil
2	810	CBS 433.97	*T. asperellum*	-	sclerotium of *Sclerotinia minor* buried in soil
3	916	CBS 226.95	*T. harzianum*	-	warcup soil plates
4	917	CBS 383.78	*T. reesei*	Salomon Islands	cellulose fabric
5	1680	IMI 206040	*T. atroviride*	Slovenia	-
6	1701	2'	*T. longibrachiatum*	Russia, Kazan	buried soils and ancient human settlements
7	3530	ATCC 13213	*T. virens*	USA	soil
8	4742	T037	*T.* cf. *harzianum*	China	herb factory
9	4803	LTR-2	*T. afroharzianum*	China	-
10	1700	1'	*T. citrinoviride*	Russia, Kazan	buried soils and ancient human settlements
11	1705	6'	*T. citrinoviride*	Russia, Kazan	buried soils and ancient human settlements
12	1708	9'	*T. citrinoviride*	Russia, Kazan	buried soils and ancient human settlements
13	2307	CBS 121302	*T. hamatum*	Italy	-

14	3020	Yarden-Israel 3807	*T. atroviride*	Israel	isolated from the marine sponge Psammocinia sp.
15	10	ICMP 3090	*T. atroviride*	New Zeeland	-
16	3027	Yarden-Israel 12907	*T. atroviride*	Israel	isolated from the marine sponge *Psammocinia* sp.
17	2663	PPRC R2	*T. atroviride*	East Africa	Hurumo woreda coffee plantation
18	408	TUB F-896	*T. virens*	Singapore	-
19	735	-	*T. virens*	Russia, Siberia	pine forest soil, lake Baikal area
20	428	TUB F-920	*T. virens*	Cambodia	Stone surface, Angkor
21	2229	39	*T. longibrachiatum*	Russia, Kazan	buried soils and ancient human settlements
22	2248	60	*T. longibrachiatum*	Russia, Kazan	buried soils and ancient human settlements
23	2282	85	*T. longibrachiatum*	Russia, Kazan	buried soils and ancient human settlements
24	661	CBS 125862	*T. parareesei*	Argentina	soil, subtropical rain forest
25	4877	LA 10	*T. harzianum* s.l. (endophyticum)	-	-
26	4878	LA 11	*T. harzianum* s.l. (endophyticum)	-	-
27	4882	IQ 11	*T. koningiopsis* sp.1	-	plants
28	4884	IQ 53	*T. koningiopsis* sp.3	-	plants

29	4885	IQ 87	*T. evansii*	-	plants
30	4886	IQ 191	*T. strigosum*	-	plants
31	4889	IQ 213	*T. strigosum*	-	plants
32	4890	IQ 246	*T. evansii*	-	plants
33	4891	PP 24	*T. harzianum* s.l. (endophyticum)	-	plants
34	4893	PP 87	*T. harzianum* s.l. (endophyticum)	-	plants
35	4894	PP 89	*T. harzianum* s.l. (endophyticum)	-	plants
36	4895	PP 136	*T. harzianum* s.l. (neotropicale)	-	plants
37	4896	MS 79	*T. spirale*	-	plants
38	4897	MS 375	*T. koningiopsis* sp.3	-	plants
39	4901	IB 50	*T. amazonicum*	-	plants
40	4902	IB 52	*T. amazonicum*	-	plants
41	116	TNS-F 237181	*T. ghanense*	Ghana	-

42	295	TUB F-781	*T.pseudoharzianum*	Viviana	soil
43	889	MA 2480	*T. rossicum*	Austria	-
44	2057	SzMC	*T. ghanense*	Hungary	*Agaricus* compost
45	2389	WJ 2337	*T.* cf. *harzianum*	Austria	-
46	2420	WJ 2858	*T. rogersonii*	Austria	-
47	2421	WJ 2861	*T. epimyces*	Austria	-
48	2432	-	*P. pallida*	Denmark	-
49	2924	SzMC A63/1/3	*T. velutinum*	Hungary	-
50	2977	-	*T. tomentosum*	Austria	soil
51	3034	-	*T. tomentosum*	Austria	soil
52	3234	-	*T. rossicum*	Austria	soil
53	4106	CBS 999.97	*T. reesei*	French Guyana	-
54	4757	CC031208-10	*T. escolopsis*	USA	nest of ants *Atta cephalotes*
55	4840	CBS c127107	*H. peltata*	USA	wood
56	202	CBS 273.78	*T. hamatum*	Columbia	-
57	230	DAOM 175924	*T. pleuroticola*	Canada	-
58	298	DAOM 230013	*T. velutinum*	Nepal	soil

59	365	CBS 433.95	*T. aggressivum*	UK	mushroom compost
60	462	G.J.S 99-159	*T.polysporum*	Australia	-
61	476	G.J.S 90-115	*T.polysporum*	USA	-
62	941	WJ 2305	*T. voglmayrii*	Austria	-
63	1592	WJ 2302	*T. voglmayrii*	Austria	-
64	1893	PPRC W6	*T. asperelloides*	Ethiopia	soil
65	2096	SzMC 3003	*T. pleuroti*	Hungary	-
66	2106	SzMC 3106	*T. aggressivum*	Hungary	-
67	2399	WJ 2457	*T. minutisporum*	Austria	-
68	2418	WJ 2850	*T. moravicum*	Austria	-
69	2657	UNISS 10-16	*T. alni*	Italy	-
70	3686	Davidson F91/06B	*T. reesei*	Brunei Darussalam	*C. cylindricus* nest in dead wood
71	3984	Marym-Egypt 42	*T.brevicompactum*	Egypt	old cultivated land
72	201	CBS 979.70	*T. coningii*	Netherlands	decaying angiosperm wood
73	306	TUB F-793	*T. coningii*	-	-
74	344	TUB F-727	*T. coningii*	Brazil	bamboo forest
75	383	CBS 347.93	*T. strictipile*	-	-

76	421	TUB F-420	*T. viride*	Thailand	soil
77	430	DAOM 230021	*T. helicum*	Thailand	-
78	495	DAOM 175931	*T. minutisporum*	Canada	-
79	527	DAOM 230004	*T. sinence*	Taiwan	-
80	631	TUB F-414	*T. gamsii*	Israel	soil
81	848	CBS 115340	*H. orientalis*	Egypt	orange tree
82	949	WJ 2380	*H. neorufa*	Austria	-
83	953	WJ 2386	*T. petersenii*	Austria	-
84	1600	CBS 118980	*T. crystalligenum*	Austria	-
85	1632	G.J.S 97-174	*T. tawa*	Thailand	-
86	4158	-	*H. novae-zelandiae*	Chile	root sample
87	4322	ER1755	*T. viridescens*	Italy	tomato
88	170	G.J.S 86-410	*T. reesei*	French Guyana	-
89	4455	GTV3	*T.harzianum*	Serbia	-
90	4804	T037	*T.guizhouense*	China	herb factory
91	4933	-	*T. reesei*	-	-
92	109	PPRI 3894	*H. orientalis*	-	-

93	202	CBS 273.78	*T. inhamatum*	Columbia	-
94	792	CBS 500.67	*H. sulphurea*	-	-
95	793	CBS 199.73	*H. alutacea*	Germany	decaying wood
96	916	CBS 226.95	*T. harzianum*	-	-
97	4455	GTV3	*T.* cf. *harzianum.*	-	-
98	4305	-	*Aspergillus nidulans*	-	wild type
99	4308	-	*A. fumigatus*	-	wild type
100	4757	-	*Escovopsis weberi*	-	-

Table 3.1.1.b. Mutant strains of *Trichoderma* and other fungi used in this study.

№	C.P.K.	Other collection codes	Species	Country	Description
1	1683	QM9414	*Trichoderma reesei*	-	Derivate of *T. reesei* QM6a
2	3790	QM 9414	*T. reesei*	-	QM 9414
3	3791	QM 9415	*T. reesei*	-	QM 9414 p*tef1*, *Δlae1* candidate O1(2)
4	3792	QM 9416	*T. reesei*	-	QM 9414 *Δlae1* candidate O2 =4324
5	3793	QM 9417	*T. reesei*	-	QM 9414 *Δlae1* candidate G2
6	4086	QM 9418	*T. reesei*	-	QM 9414 p*tef1 Δlae1* candidate M2-3
7	4496	P1	*T. atroviride*	-	candidate L4 *tef1*:*lae1*
8	4497	P1	*T. atroviride*	-	candidate 133 *Δlae1*
9	4498	-	*T. atroviride*	-	P1 control for the transformants
10	4305	-	*Aspergillus nidulans*	-	wild type
11	4307	-	*A. nidulans*	-	*gpdA* (p)::*lae1*, *pryoA*
12	4308	-	*A. fumigatus*	-	wild type

13	4309	-	*A. fumigatus*	-	*ΔlaeA*::A parasiticus *pyr*G *pyr*G1;
14	4306	-	*A. nidulans*	-	methG1; *ΔlaeA*::methG, veA+;
15	4604	QM 9414	*T. reesei*	-	Q2V2 *Δvel1*
16	4605	QM 9414	*T. reesei*	-	Q2V1 *Δvel1*
17	4916	916L29	*T. harzianum*	-	*lae1*OE
18	4917	916L30	*T. harzianum*	-	*lae1*OE
19	4918	916L34	*T. harzianum*	-	*lae1*OE
20	4919	916X14	*T. harzianum*	-	*xyr1*OE
21	4920	916X15	*T. harzianum*	-	*xyr1*OE
22	4921	916X21	*T. harzianum*	-	*xyr1*OE
23	4922	916XL1	*T. harzianum*	-	*xyr1*OE and *lae1*OE
24	4923	916XL2	*T. harzianum*	-	*xyr1*OE and *lae1*OE
25	4924	4455L39	*T.* cf. *harzianum*	-	*lae1*OE
26	4925	4455L40	*T.* cf. *harzianum*	-	*lae1*OE
27	4926	4455L41	*T.* cf. *harzianum*	-	*lae1*OE

28	4927	4455X23	*T.* cf. *harzianum*	-	*xyr1*OE
29	4928	4455X25	*T.* cf. *harzianum*	-	*xyr1*OE
30	4929	4455X27	*T.* cf. *harzianum*	-	*xyr1*OE
31	4930	4455XL8	*T.* cf. *harzianum.*	-	*xyr1*OE and *lae1*OE
32	4931	4455XL9	*T.* cf. *harzianum.*	-	*xyr1*OE and *lae1*OE
33	4932	4455XL17	*T.* cf. *harzianum*	-	*xyr1*OE and *lae1*OE

3.2. Semi-high throughput method for the assessment of fungal response to illumination

It was previously shown that *T. atroviride* C.P.K. 1680 is strongly photostimulated (Friedl et al. 2008). Whereas *T. reesei* C.P.K. 917 (QM6a) is fully insensitive to illumination (Druzhinina et al., 2010). Some other species such as *T. harzianum* C.P.K. 916 were showing low response on the light stimulation (Marzouk et al. 2011). Based on previous results it was decided to use these three fungi strains as model organisms to design semi-highthroughput method for the assessment of fungal response to the blue light illumination.

3.2.1. Optimization of cultivation conditions

In the optimization part of the experiment several types of mediums were used to observe changes in the growth rates of different fungal strains: Potato Dextrose Agar (PDA 39.0 g/l); Malt extract agar (Malt extract 30.0 g/l, Agar 20.0 g/l); Lysogeny Broth (Peptone 10.0 g/l, Yeast extract 5.0 g/l, NaCl 10.0 g/l, Agar 1.5%); Synthetic Nutrient - Poor Agar **(**KH2PO4 1.0 g/l, NH4NO3 2.0 g/l, MgSO4*7H2O 0.5 g/l, KCl 0.5 g/l, Glucose 0.2 g/l, Sucrose 0.2 g/l, Agar 20.0 g/l) and Synthetic Nutrient - Rich Agar (composition of medium the same as by normal SNA except Glucose 1.0 g/l, Sucrose 1.0 g/l).

Additionally carbon sources in SNA and SNA rich were replaced to Glycerol, Glucose, Fructose, Mannose and Xylose, respectively, each in the concentrations from 2 till 9%. Experiments were done on 96 (12x8) sterile well plates (Sigma-Aldrich, Hanover, Germany). Volume of each sample was equal to 200 µl. All plates were cultivated at 25 ºC temperature.

The influence of the blue light on the growth of different *Trichoderma* species was studied using Phytagel (FF Inoculating Fluid, Biolog) and salts from SNA rich medium with Glucose as a carbon source (2%). Strains were pregrown for 5 days on MEX plates. Each strain was inoculated in two well plates, where one was exposed to 12h light cycle and the other was incubated in darkness.

The first 12h cycle was starting with 12 hours or darkness because spores are germinating better without light.

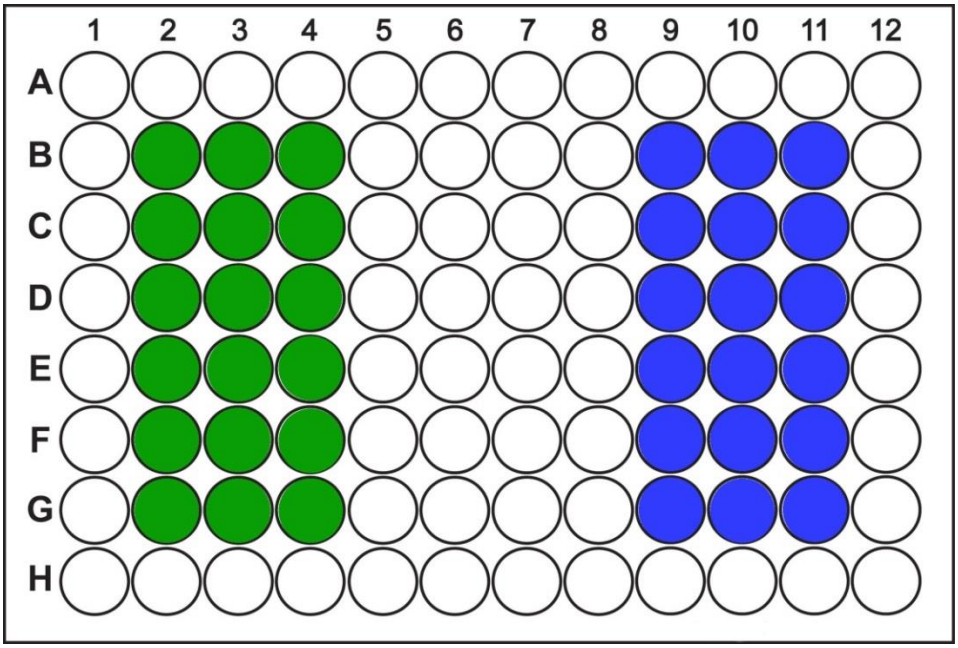

Figure 3.2.1 Schematic view of 96 well plates. Different colors are showing location of the two strains during the experiment

3.2.2. Preparation of the medium in four steps:

- 10 ml of Phytagel (FF Inoculating Fluid, Biolog) were added to sterile biolog tubes.
- High concentrated salt solution with Glucose * (or without Glucose depending on the experiment) was prepared and added 1 ml of it to the tube with phytagel (previously prepared).
- 1 ml of salt solution (that was added to 10 ml of phytagel) included: KH_2PO_4 0.01 g; NH_4NO_3 0.02 g; $MgSO_4*7H_2O$ 0.005 g; KCl 0.005 g; Glucose (Carbon source) 0.2 g (2%).

***** Afoot note: For the experiment it is necessary to prepare 100 ml of the solution with salts and Glucose. 50% glucose solution will be used for this experiment. Glucose solution should be filtered with 0.2 nm filter. Carbon source will be added to the medium after autoclaving to avoid caramelization of Glucose. It is possible to calculate that 40 ml of glucose 50% will be

used for 100 ml. Based on that - KH_2PO_4 (1 g), of NH_4NO_3 (2 g), $MgSO_4*7H_2O$ (0.5 g) and KCl (0,5 g) will be dissolved in 60 ml of water and autoclaved. After that 40 ml of glucose will be added till 100 ml volume of the solution.

- Chloramphenicol with concentration 1g/l to the medium was added.

3.2.3 Procedure of the experiment

Strains were pregrown for 5 days on MEX plates exposed to light to induce the sporulation. Spores were added to the medium that included Phytagel, salt mix, glucose and antibiotic. Turbidity of spore solution was measured (75% of turbidity). Each strain was inoculated in two well plates, where one was exposed to 12h light cycle and the other was incubated in darkness. The first 12h cycle was starting with 12 hours or darkness.

Results were measured with Biolog and Glomax microplate reader machines (750 nm). Measurements were done each 24h for maximum 168h. Time periods for measurements were 0, 24, 48, 72, 96 and 168 hours. Plates were photographed after 48, 72, 96 and 168 hours.

3.3. Statistical data evaluation

All measurements were done with Biolog (Hayward CA, USA) and Glomax (Promega, Fitchburg, USA) microplate reader machines at 750 nm. Results that were obtained from both readers showed similar outcomes. There are some differences in structures of readers that effect on ways of measuring samples. Biolog reader is a time-proved and reliable instrument with one sensor optical system. On the other hand Glomax reader represents another generation of instruments that are using multi sensor system and could provide us with more precise numbers. After the comparison of the results it was decided to work subsequently with the data set obtained with Biolog device, because of the lower variability of the samples compared to Glomax data.

All data from experiments were assembled in one analytical matrix. This matrix was processed with Statistica 6.1 program (StatSoft Inc, Tulsa). All data were first subjected to descriptive statistical evaluations (mean, minimum, maximum and standard deviation values) and checked for outliners. Secondly, we used cluster analysis to detect possible groupings in our results. The term cluster analysis encompasses a number of different algorithms and methods for grouping objects of similar kind into respective categories. Methods that were taken as a basis for an analysis were described in previous researches (Friedl et al. 2008).

Hypotheses that were assumed on the basis of experimental data were tested and confirmed by the analysis of variance (ANOVA). This is accomplished by analyzing the variance by portioning the total variance in to the component that is due to true random error (within - group) and the components that are due to differences in means. These latter variance components are than tested for statistical significance. In a simple example with only two groups the same result can be achieved with a *t*-test, However, ANOVA is a much more flexible technique that can be applied to much more complex research issues. Usually in a typical experiment, many factors are taken in to account. In ANOVA it is possible to test each factor while controlling for all the others. Another advantage of ANOVA is that it allows to detect interaction effects between variables and therefore to test complex hypotheses ("Analysis of Variance" 2014).

3.3.1. Phylogenetic analysis

For phylogenetic analysis DNA sequences from Atanasova et al. (2013) which corresponded to the strains used in this study were aligned with the ClustalX program (version 1.81) and visually verified with GeneDoc software (version 2.6). Phylogenetic analysis was performed using the maximum parsimony method using a heuristic search (*n*=1000) with the random addition of sequences and the TBR tree-swapping algorithm. The reliability of the obtained clades was tested by 500 bootstrap replications.

For the representation of the analytical results of this work the phylogenetic tree is based on the partial *rpb2* gene encoding the RNA polymerase II subunit (RPB2) was build. This subunit is the second-largest subunit that in combination with at least two other polymerase subunits forms a structure within the polymerase that maintains contact in the active site of the enzyme between the DNA template and the newly synthesized RNA in eukaryotic cells.

3.4. Gene expression analysis

3.4.1. Culturing conditions of strain for RNA isolation.

T. parareesei (C.P.K. 717), *T. harzianum* (C.P.K. 916) and *T. atroviride* (C.P.K. 1680) were selected to be tested for expression of *phr1*, *nox1* and *lae1* genes because of the availability of sequenced genomes. The strains were cultivated on 16 ml of solid MEX medium covered with cellophane. Chloramphenicol antibiotic with the 0.5 g/l concentration was added to the medium to avoid possible bacterial contaminations. The strains were cultivated as described above (25ºC, 12h of constant light cycles at 2600 lux or darkness).

3.4.2. Samples collection and RNA isolation

Mycelium for the RNA extraction was collected after 60h to prevent high amounts of spores in the samples. All samples were frozen in liquid nitrogen immediately after the sampling and were stored in -80ºC. Total RNA was isolated using RNeasy Plant Mini Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). All materials that were used in the experiment were treated with diethyl pyrocarbonate (DEPC) (Sigma-Aldrich, Hanover, Germany) to avoid degradation of RNA by RNases. The concentration of the RNA was determined with NanoDrop spectrophotometer (Thermo Scientfic, USA). Presence of RNA was confirmed with electrophoresis on 1% agarose gel (90V, 400mA and 60 min).

3.4.3. Complementary DNA (cDNA) synthesis

The RNA sample (1 µg) was added to an RNAse-free tube and the 10x reaction buffer with MgCl2 (1 µl), DEPC-treated Water (till 9 µl) and Deoxyribonuclease I (1 µl) were added. The mixture was incubated at 37°C for 30 min. After that 25mM EDTA (1 µl) was added and incubated at 65°C for 10 min. The prepared RNA template was used for reverse trancriptase (Fermentas Kit). All steps were performed on ice. To the total RNA sample Oligo (dT)18 primer 0.5 µg/µl (1 µl) was added, mixed gently and centrifuged briefly. The mixture was incubated at 70°C for 5 min, then chilled on ice and centrifuged briefly. The tube was placed on ice and 5x reaction buffer (4 µl), RiboLock Ribonuclease inhibitor (1 µl), 10mM dNTP mix (2 µl) were added. After the incubation at 37°C for 5 min RevertAid H Minus M-MulV RT (1µl) was added. In the final step of the procedure the sample mixture was incubated at 42°C for 60 min. The reaction was stopped by heating at 70°C for 10 min.

The final concentration of the cDNA was determined with NanoDrop spectrophotometer (Thermo Scientfic, USA). Presence of cDNA was confirmed with electrophoresis on 1% agarose gel (90V, 400mA and 60 min).

3.4.4. Primer design

Alignments of *phr1*, *nox1* and *lae1* genes for *T. parareesei* (C.P.K. 717), *T. hrzianum* (C.P.K. 916) and *T. atroviride* (C.P.K. 1680) were taken from JGI Genom portal (http://genome.jgi-psf.org/). These alignments were analyzed with GenDoc and ClustalX software. All sequences were compared manually and automatically (Beacon design software) to find the regions that were strictly conserved in all three strains. The regions were then scanned for proper primers using the NCBI primer design tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) to design primers for RT-PCR. As it was not possible to find a consensus primer for *T. atroviride* (C.P.K. 1680), it was decided to design a separate pair of primers just for this species. List of the designed primers with additional technical information is shown in supplementary materials (S5).

3.4.5. Reverse transcription polymerase chain reaction (RT-PCR)

Synthesized cDNA, diluted in 50 times, was amplified with specifically designed primers for three genes (*phr1*, *nox1* and *lae1*) using the following protocol (S2) with a gradient of temperatures to determine the optimal temperature for each gene.

PCR program:

Hot start	95ºC		
Denaturation	95 ºC	2 min	
Annealing	51.5-62.5 ºC	1 min	30 cycles
Extension	72 ºC	3 min	
Final extension	72 ºC	10 min	

T_m was optimized for each pair of primers using genomic DNA of *T. parareesei* (C.P.K. 717), *T. harzianum* (C.P.K. 916) and *T. atroviride* (C.P.K. 1680) for the experiment. Results are shown below (Fig 3.4.5.1-3.4.5.3). All PCR protocols that were used are presented in supplementary materials (Protocol № S2-S4) of this thesis.

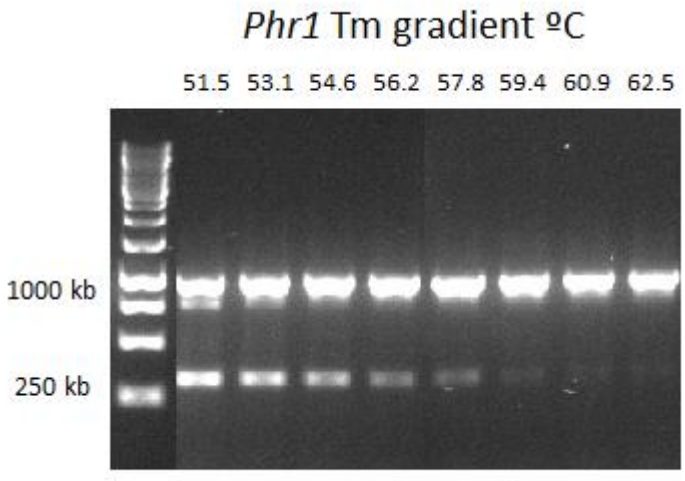

Figure 3.4.5.1 is showing T_m optimization of for *phr1* gene using the temperature gradient and strain *T. atroviride* (C.P.K. 1680).

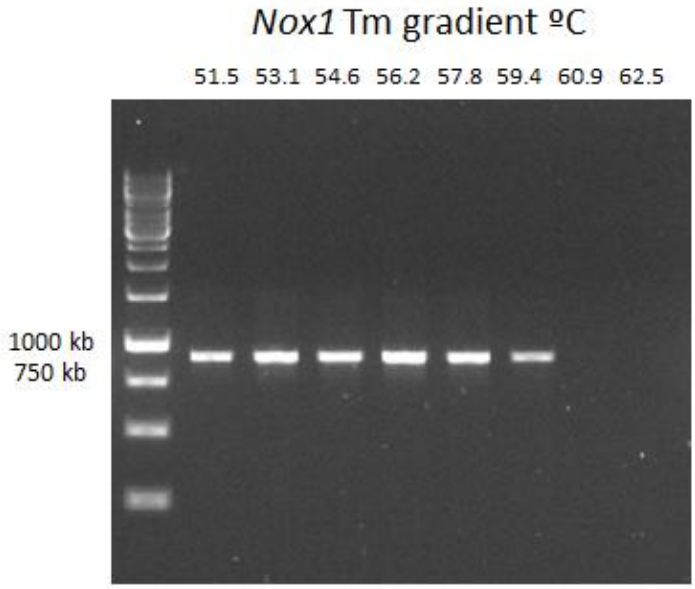

Figure 3.4.5.2 is showing T_m optimization of for *nox1*gene using the temperature gradient and strain *T. longibrachiatum* (C.P.K. 1701).

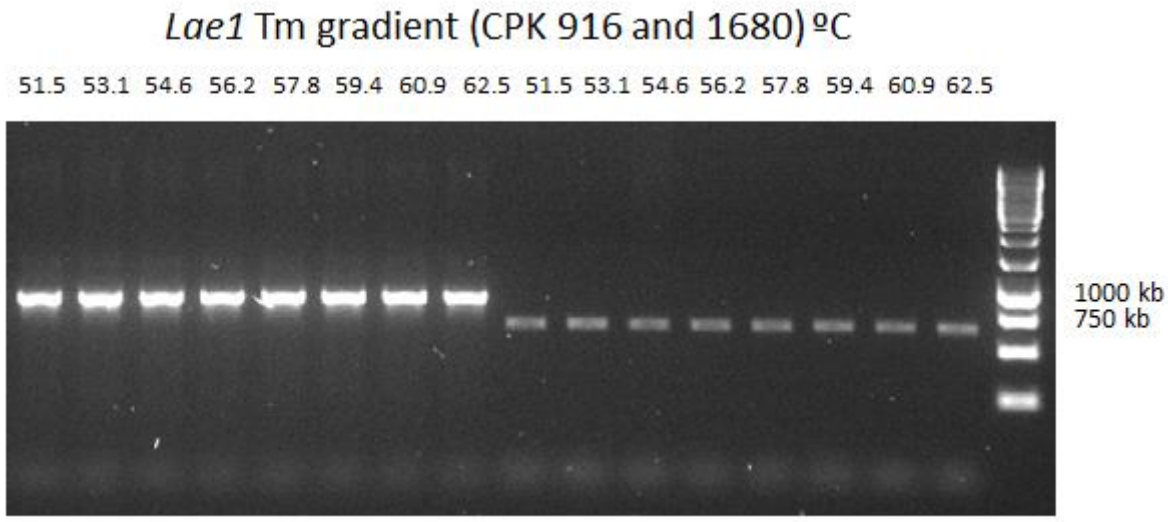

Figure 3.4.5.3 is showing T_m optimization of for *phr1* gene using the temperature gradient and strain *T.atroviride* (C.P.K. 1680).

The analysis of gel pictures showed that the best T_m for *phr1* and *lae1* genes is 63 ºC and for *nox1* optimized Tm is 56 ºC. The Tm for each gene was used for expression analysis using the protocol described above. Expression of the light-dependent genes was confirmed with electrophoresis on 1.5% agarose gel (80V, 200mA, 40 min).

4. Results

4.1. The blue light influence on mycelial growth of *Trichoderma* strains.

All data were collected and represented in the matrix that was processed by "Statistica 6.1" software. The matrix has represented 98 wild-type strains from 54 species and 26 clades of *Trichoderma* and 2 wild-type strains of *Aspergillus* and one wild-type strain of *Escovopsis*. There were 29 species represented only by one strain, 16 species represented by 2 strains and 11 species represented by two or more strains. The matrix contains the information about growth rates of *Trichoderma* strains measured after 48, 72 and 96 hours in dark and light conditions. Measurements that were made after 24 and 168 hours were not taken into account because data from this time points were on the lag- or stationary growth phases respectively.

In the first step of the data analysis cluster analysis was performed. Fig. 4.1.1 shows that closely related strains (for example, *T. harzianum* sensu lato is marked with green color and section *Trichoderma* with red color) do not cluster together based on their growth pattern in light and darkness. All strains are divided in two big clusters or four smaller. Strains are not represented in the phylogenetic (evolutionary) order. This explorative analysis does not give a precise answer to the question about the effect of light on the *Trichoderma* genus in general.

Fig. 4.1.2 shows the phylogramm of *Trichoderma* strains. The small diagrams on the right side of the figure are presenting four groups and carrying data that represent differences between measured optical densities (750 OD) of strains in dark and light conditions. Thus, all positive values indicate phostimulation while negative values correspond to photoinhibition. All measurements were made after 48, 72 and 96 hours that are shown by different colors on the figure. Some species like *T.* cf. *endophyticum, T.* cf. *neurotropicale, T. virens, T. parareesei, T. longibrachiatum, T. atroviride* and *T. koningiopsis* sp. are showing high positive results on light illumination (ANOVA, $P<0.05$). On the other hand there are several strains which growth is inhibited by the blue light. Genetically close

strains in each cluster are showing mixed results that can lead to a conclusion that *Trichoderma* genus is neither photostimulated, nor photoinhibited genus.

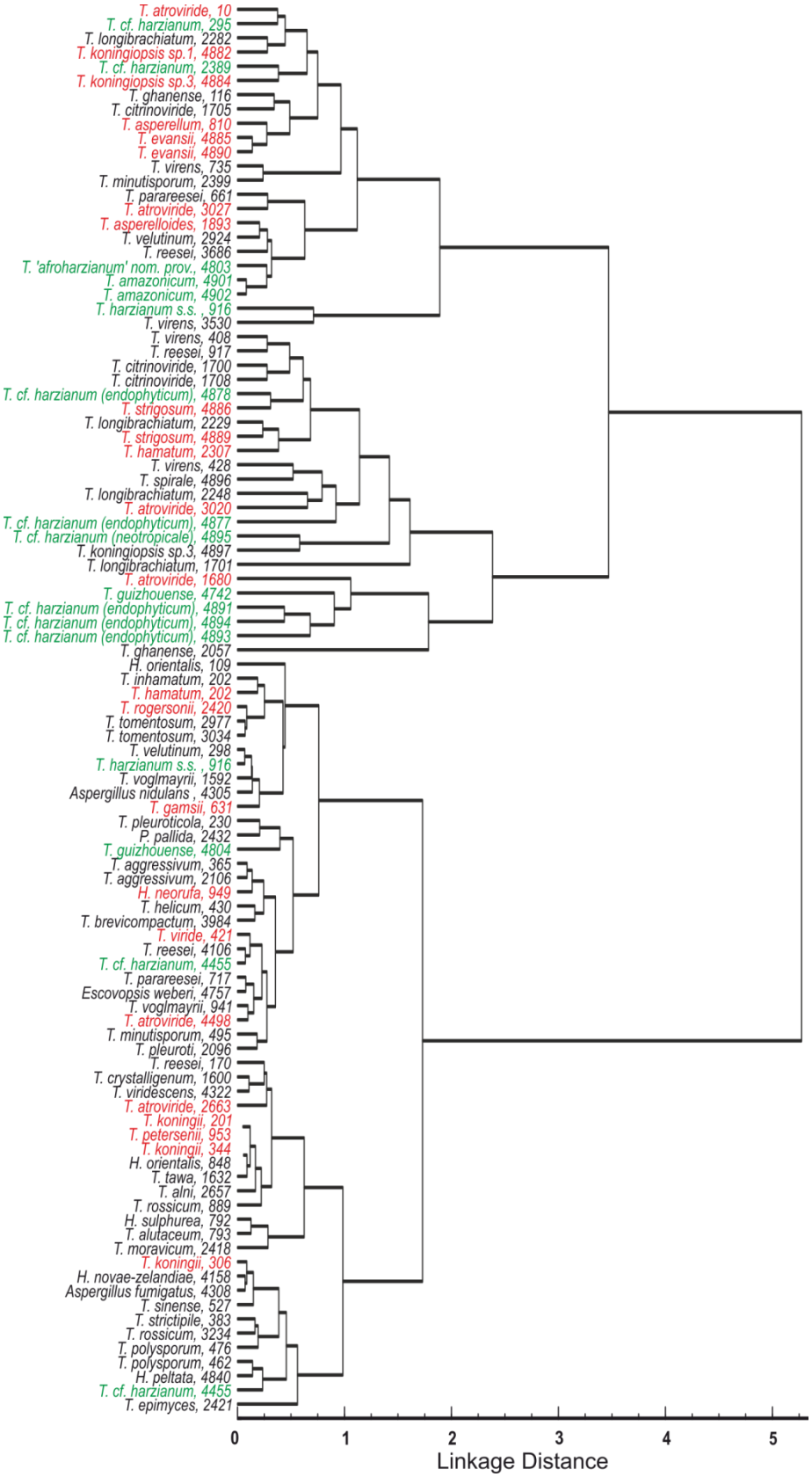

Figure 4.1.1. Cluster analysis shows that closely related strains (*Harzianum* Clade in green and Section *Trichoderma* in red). A single line corresponds to one strain used in the experiment.

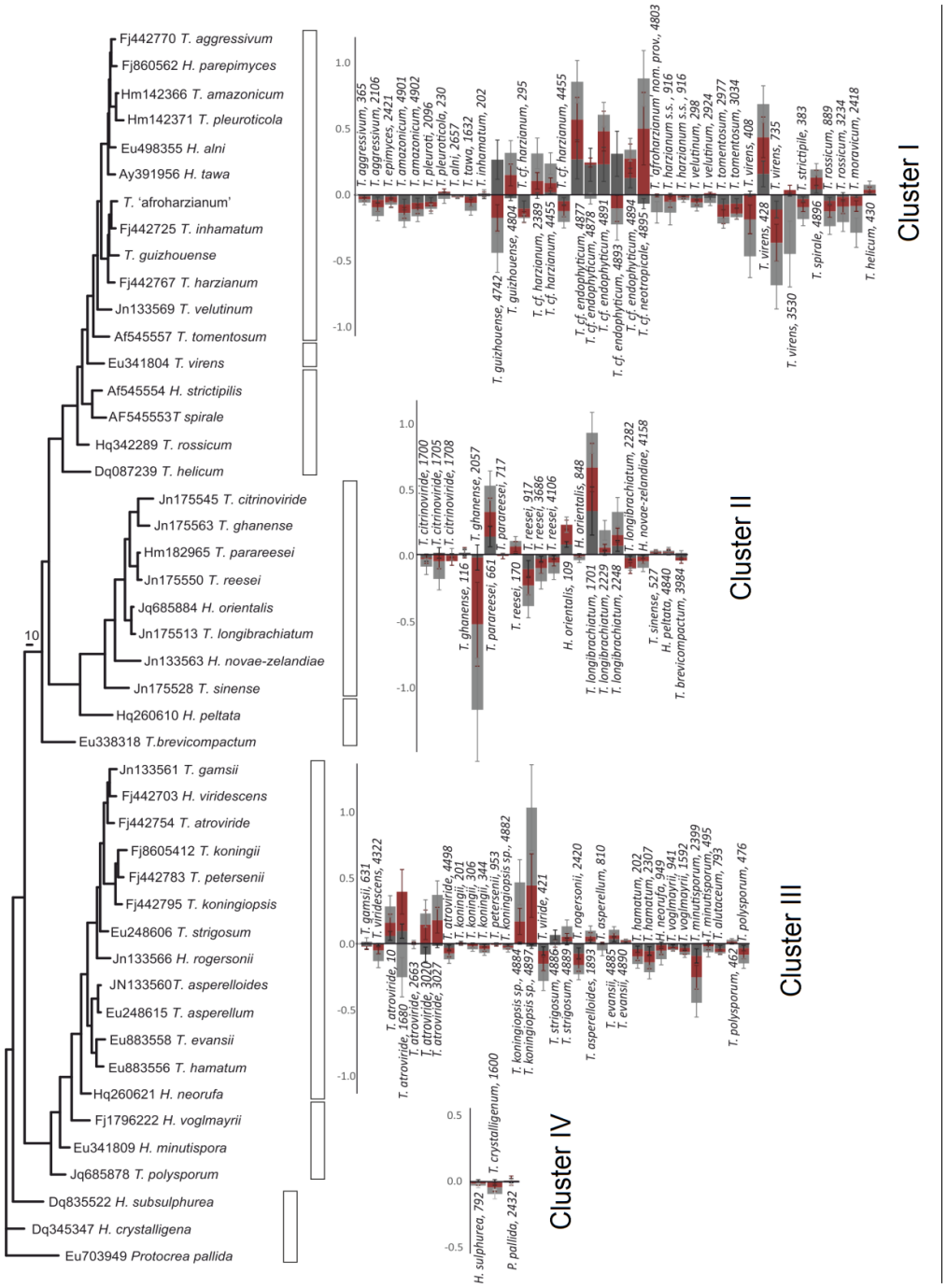

Figure 4.1.2. Left side: shows the maximum parsimony phylogenetic tree of *Trichoderma* strains used in the experiment. Bootstrap support based on 500 numberso of replicates is provided in the supplementary material (S8) and in Atanasova et al. 2013. The scale on the tree represents 10 nt substitutions or indels. It is based on the *rpb2* gene encoding RNA polymerase II subunit (RPB2). A single line corresponds to one strain used in the experiment. Right side: represents 4 separate groups and the differences between growth rates of strains used in the experiment under the blue light stimulation (12 hours every 24 hours) and the darkness. The positive scale represents strains that are photostimulated. The negative scale represents strains that are photoinhibited. Different colors such as dark grey, reddish and light grey are showing 48, 72 and 96 hours measurements time points, respectively.

To confirm the hypothesis that *Trichoderma* genus in general is photosensitive we have used Factorial ANOVA analysis. Growth rates were chosen as the dependent variable and light conditions, time points, strains and species were chosen as categorical predictors. Results of the analysis are showing that light is not a significant factor (ANOVA, Current effect: $F(1, 2004)=1.4135$, $p=0.23462$) for *Trichoderma* genus in general.

In the next step we have looked on four (I-IV respectively) clades (*Harzianum* clade, *Longibrachiatum* clade, Section *Trichoderma* clade and single lineages) that are represented on the Fig. 4.1.2 and tried to confirm the hypothesis that the light is significant for each of the clades separately. Analysis showed that for *Harzianum* section (ANOVA, Current effect: $F(1, 588)=0.4245$, $p=0.83684$), *Longibrachiatum* section (ANOVA, Current effect: $F(1, 336)=0.008$, $p=0.929206$), *Trichoderma* section (ANOVA, Current effect: $F(1, 462)=0.0390$, $p=0.532759$) and additional section with other species (ANOVA, Current effect: $F(1, 30)=0.074$, $p=0.787588$) light factor is not significant.

However, it is notable (Fig. 4.1.2) that phylogenetically related species in each of four clusters are represented by photosensitive and photoinhibited species at the same time.

The list of species represented by two or more strains that are showing constant photoinhibition is indicated in the Table below (Tab. 4.1.1). Information that confirms or disproves the hypothesis that light is a significant factor for the species is in the second column. Additional materials presenting measurements of growth rates for this species after 48, 72 and 96 hours are available in supplementary materials (S6).

Table 4.1.1 The confirmation of the hypothesis that light is a significant factor for the photo inhibited species (bold font for significant confirmations).

Photoinhibited species	Test of the hypothesis about the effect of light on species
T. agressivum **(C.P.K.s 365, 2106)**	**(ANOVA, Current effect: F(1, 30)=79.53, p=0.00)**
T. amazonicum **(C.P.K.s 4901, 4902)**	**(ANOVA, Current effect: F(1, 30)=69.60, p=0.00)**
T. velutinum **(C.P.K.s 298, 2924)**	(ANOVA, Current effect: F(1, 30)=0.840, p=0.366755)
T. tomentosum **(C.P.K.s 2977, 3034)**	**(ANOVA, Current effect: F(1, 30)=181.90, p=0.00)**
T. rossicum **(C.P.K.s 889, 3234)**	**(ANOVA, Current effect: F(1, 30)=0.15,2488 p=0.00049)**
T. citrinoviride **(C.P.K.s 1700, 1705, 1708)**	**(ANOVA, Current effect: F(1, 48)=4.92, p=0.031280)**
T. hamatum **(C.P.K.s 202, 2307)**	(ANOVA, Current effect: F(1, 30)=0.8278, p=0.370172)
T.voglmayrii **(C.P.K.s 941, 1592)**	(ANOVA, Current effect: F(1, 30)=4.072, p=0.052627)
T. minutisporum **(C.P.K.s 495, 2399)**	(ANOVA, Current effect: F(1, 30)=2.4434, p=0.128512)
T. strigossum **(C.P.K.s 4886, 4897)**	(ANOVA, Current effect: F(1, 30)=3.55, p=0.069147)
T. koningii **(C.P.K.s 201, 306, 344)**	**(ANOVA, Current effect: F(1, 48)=4.105, p=0.048344)**

Interestingly, that there is no examples of photostimulated or photoneutral species that were tested based on two or more strains.

Some of these species (represented by two or more strains) are showing versatile results. The list of these species represented in the table below (Tab. 4.1.2). Information that confirms or disproves the hypothesis that light is a significant factor for the species is in the second column. Additional materials presenting measurements of growth rates for this species after 48, 72 and 96 hours are available in supplementary materials (S7).

Table 4.1.2 The confirmation of the hypothesis that light is a significant factor for the versatile species (bold font for significant confirmations).

Versatile species	Test of the hypothesis about the effect of light on species
T. guizhouence **(C.P.K.s 4742, 4804)**	(ANOVA, Current effect: F(1, 30)=0.0156, p=0.901437)
T. **cf.** ***harzianum*** **(C.P.K.s 295, 2389, 4455)**	(ANOVA, Current effect: F(1, 66)=0.0746, p=0.785657)
T. **cf.** ***endophyticum*** **(C.P.K.s 4877, 4878, 4891, 4893, 4894)**	**(ANOVA, Current effect: F(1,84)=9.730, p=0.002484)**
T. virens **(C.P.K.s 408, 428, 735, 3530)**	(ANOVA, Current effect: F(1, 66)=2.107, p=0.151378)
T. ghanense **(C.P.K.s 116, 2057)**	**(ANOVA, Current effect: F(1, 30)=4.8484, p=0.0355)**
T. parareesei **(C.P.K.s 661, 717)**	(ANOVA, Current effect: F(1, 30)=3.5051, p=0.070958)
T. reesei **(C.P.K.s 170, 917, 3686, 4106)**	(ANOVA, Current effect: F(1, 66)=0.7240, p=0.397917)
T. orientalis **(C.P.K.s 109, 848)**	(ANOVA, Current effect: F(1, 30)=0.2911, p=0.593505)
T. longibrachiatum **(C.P.K.s 1701, 2229, 2248, 2282)**	**(ANOVA, Current effect: F(1, 66)=4.743, p=0.033)**
T.atroviride **(C.P.K.s 10, 1680, 3020, 3027)**	(ANOVA, Current effect: F(1, 102)=0.4350, p=0.5110)
T.polysporum **(C.P.K.s 462, 476)**	**(ANOVA, Current effect: F(1, 30)=7.378, p=0.010851)**

All these results are underlying the efficiency of the developed method for the assessment of fungal response to the illumination. It is possible to make a conclusion that generally *Trichoderma* genus is photoneutral, but can make a statement that some of the species have properties to be photostimulated or photoinhibited.

4.2. The blue light influence on mycelial growth of *Aspergillus* and *Escovopsis* strains.

Results for *Aspergillus* and *Escovopsis* fungi strains were also analyzed.

A. fumigatus C.P.K. 4308 (ANOVA, Current effect: F(1, 12)=107.39, p=0.00) showed photoinhibited results and *A. nidulans* C.P.K. 4305 (ANOVA, Current effect: F(1, 12)=0.429, p=0.524948) was photoneutral (Fig. 4.2.1).

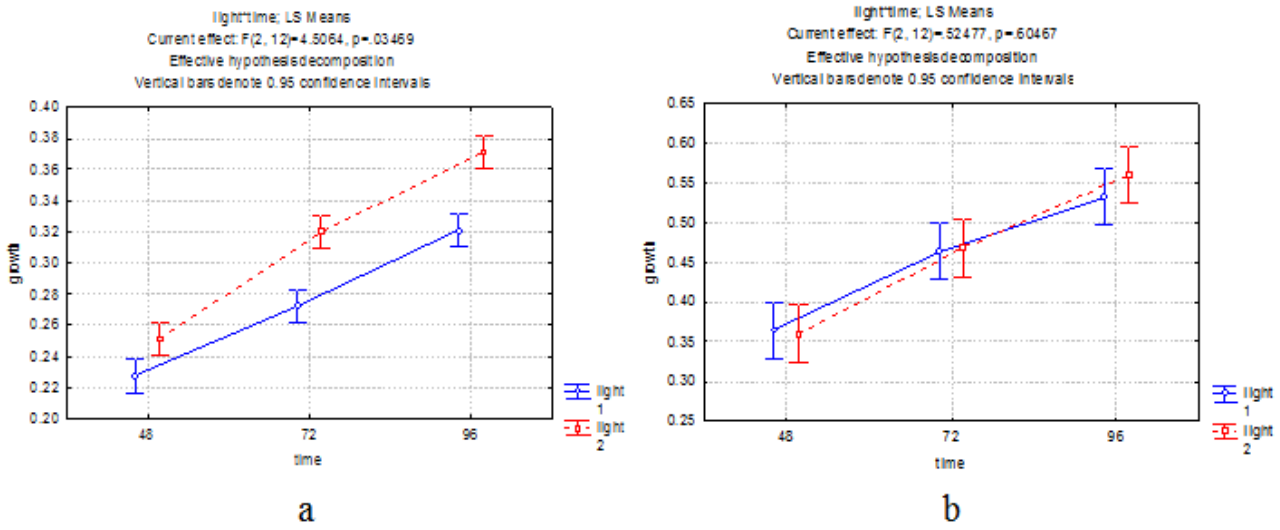

Figure 4.2.1. The measurements of growth rates of *A. fumigatus* C.P.K. 4308(a) and *A. nidulans* C.P.K. 4305 (b) in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

As for *Escovopsis weberi* strain C.P.K. 4757 it was shown a minimal although statistically significant photoinhibition (ANOVA, Current effect: F(1, 12)=33.19, p=0.00) (Fig. 4.2.2).

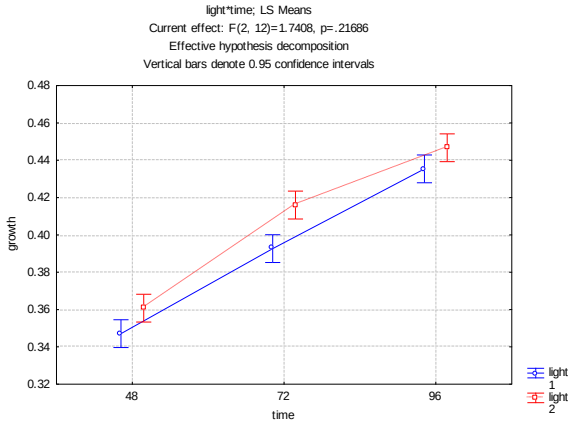

Figure 4.2.2. The measurements of growth rates of *E. weberi* C.P.K. 4757 in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

4.3. The blue light influence on mycelial growth of wild type strains and their mutants.

One of the interesting cases shows that *T. reesei* wild strains (C.P.K.s 170, 917, 3686, 4106) having the tendency to be photoinhibited (Fig. 4.3.1). This assumption is confirmed by testing the hypothesis about the significance of light for the species (ANOVA, Current effect: $F(1, 48)=166.10$, $p=0.00$). On the other hand mutant strains with deletion of *lae1* gene (C.P.K.s 3792 and 3793 with *Δlae1*) are showing positive photostimulation (ANOVA, Current effect: $F(1, 12)=0.28$, $p=0.509$) (Fig. 4.1.8). Moreover *T. reesei* mutant (C.P.K. 4604) with the knocked out *Δvel1* gene presents higher photoinhibition levels in compare to wild strains (Fig. 4.3.1). The knockout of *vel1* gene by *T. virens* cause impaired conidiation on solid medium and production of chlamydospores on liquid medium, thus underling the role of this gene in morphogenesis (V. K. Gupta and Ayyachamy 2012).

Aspergillus species (wild type C.P.K.s 4308, 4305 and mutants C.P.K.s 4309, 4306, 4307) have shown no photoresponce to the blue light spectrum. The hypothesis of the significance of the light for *Aspergillus* species was checked and it was confirmed that light is not significant for this fungi (ANOVA, Current effect: $F(1, 30)=9.19$, $p=0.5964$).

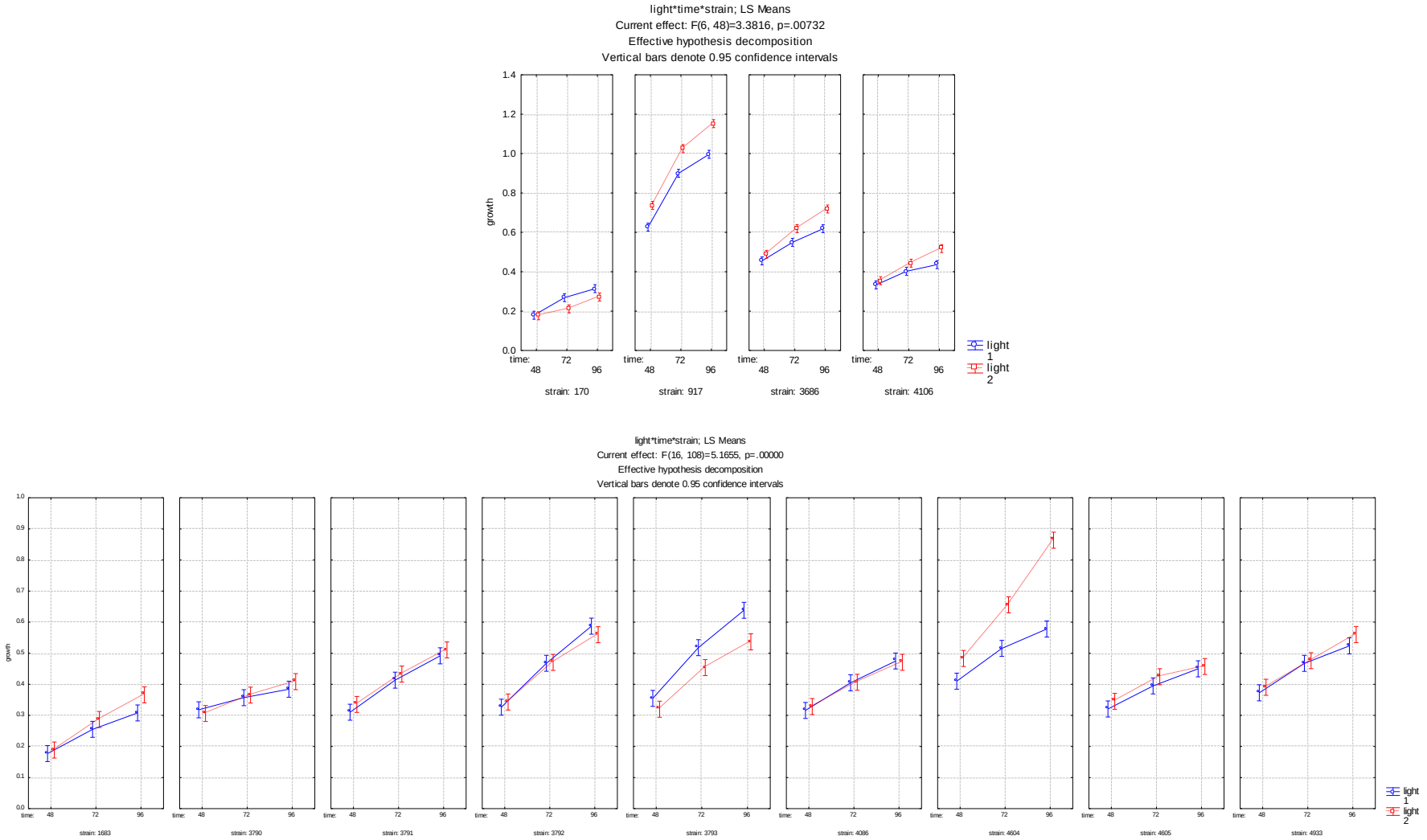

Figure 4.3.1. Measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours for *T. reesei* (wild strains and mutants)

4.4. The blue light influence on conidiation of *Trichoderma*

Two strains of *T. ghanense* have shown versatile growth responses to the illumination: while *T. ghanense* C.P.K. 116 strain was photoneutral at all-time points (ANOVA, Current effect: F(2, 12)=.06980, p=.93296), the *T. ghanense* C.P.K. 2057 strain was largely photoinhibited (ANOVA, Current effect: F(2, 12)=6.5010, p=.01222). This pattern did not correspond to the conidiation that has been estimated at 72 hours of incubation. Photoinhibited strain C.P.K. 2057 has shown higher rates of conidiation in light conditions (Fig. 4.4.1).

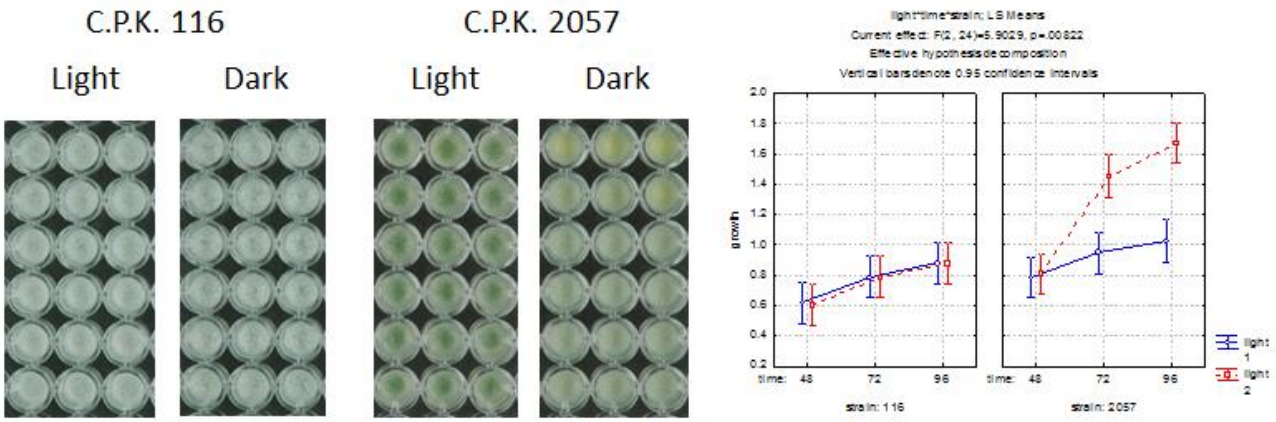

Figure 4.4.1 Left part shows the sporulation of *T. ghanense* after 72 hours under blue light illumination and darkness. Right part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours, respectively.

Four strains of *T. longibrachiatum* have shown different response to the blue light stimulation: three strains C.P.K 1701 (ANOVA, Current effect: F(2, 12)=.99001, p=.39998), 2229 (ANOVA, Current effect: F(2, 12)=15.962, p=.00042) and 2248 (ANOVA, Current effect: F(2, 12)=3.1990, p=.07700) were photostimulated, while C.P.K. 2282 (ANOVA, Current effect: F(2, 12)=21.707, p=.00010) was photoinhibited. This data were confirmed with strains pictures on the plates that have shown higher rates of conidiation under the photostimulation (Fig. 4.4.2).

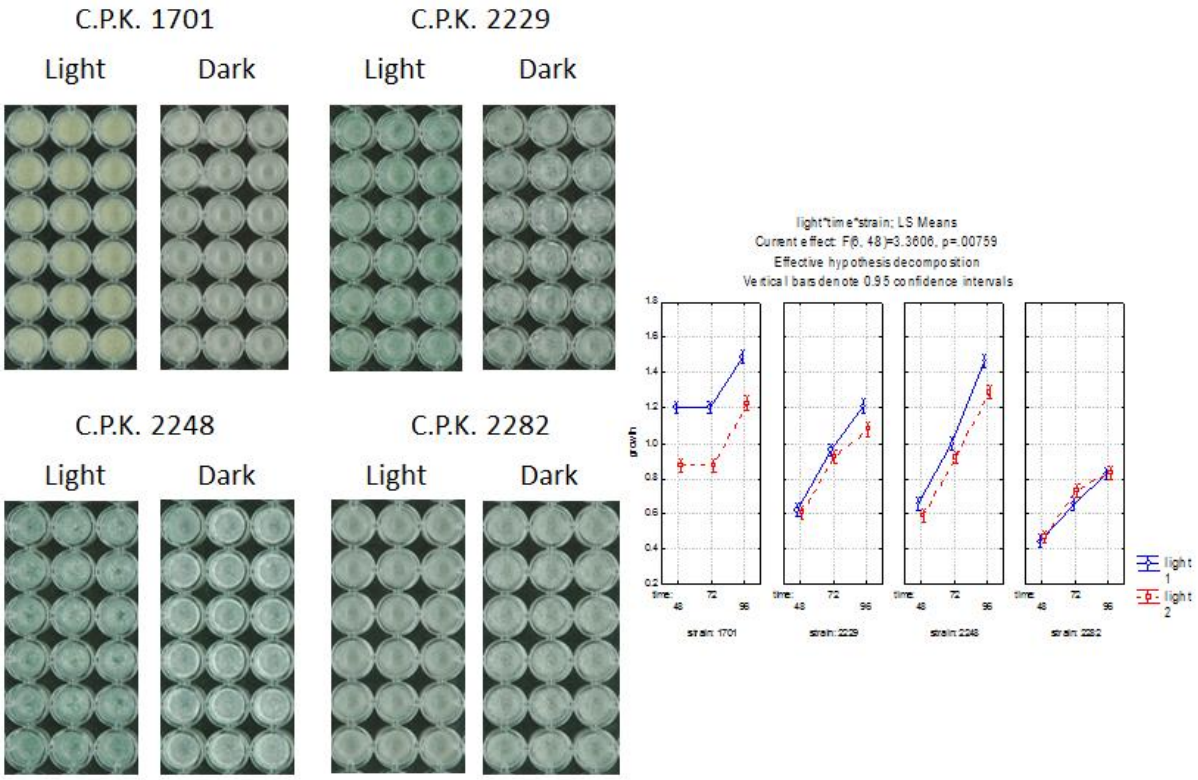

Figure 4.4.2. First part shows the sporulation of *T. longibrachiatum* after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

Two strains of *T. vellutinum* C.P.K.s 298 (Current effect: F(2, 12)=.32351, p=.72973) and 2924 (ANOVA, Current effect: F(2, 12)=63.704, p=.00000) have shown photoinhibition results. This data is confirmed by low conidiation rates showed on the picture (Fig.4.4.3).

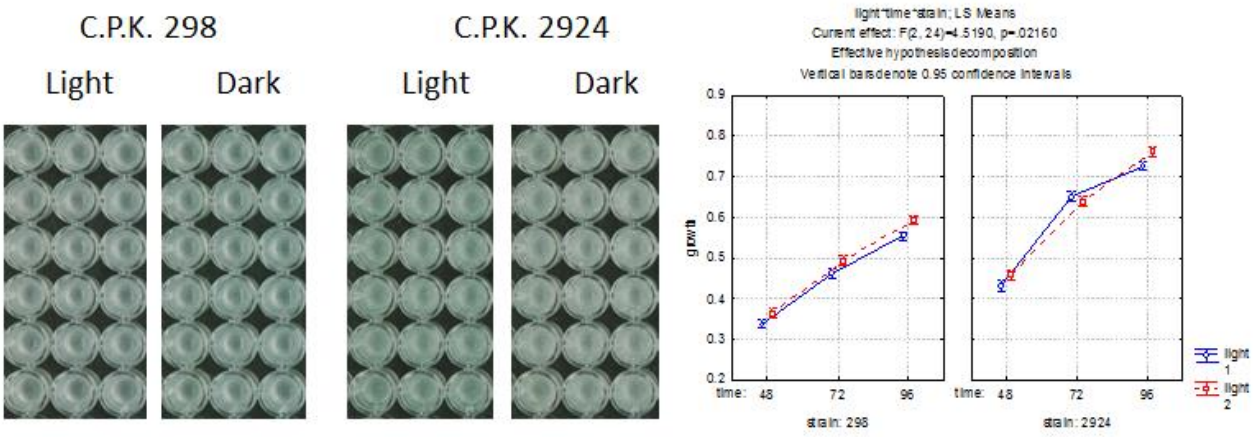

Figure 2.4.3. First part shows the sporulation of strains of *T. velutinum* after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

T. harzianum sensu stricto is represented by one strain C.P.K. 916 (ANOVA, Current effect: F(2, 30)=.01841, p=.98177). This strain was photoneutral on all time points. Conidiation rates were also on the same level (Fig. 4.4.4).

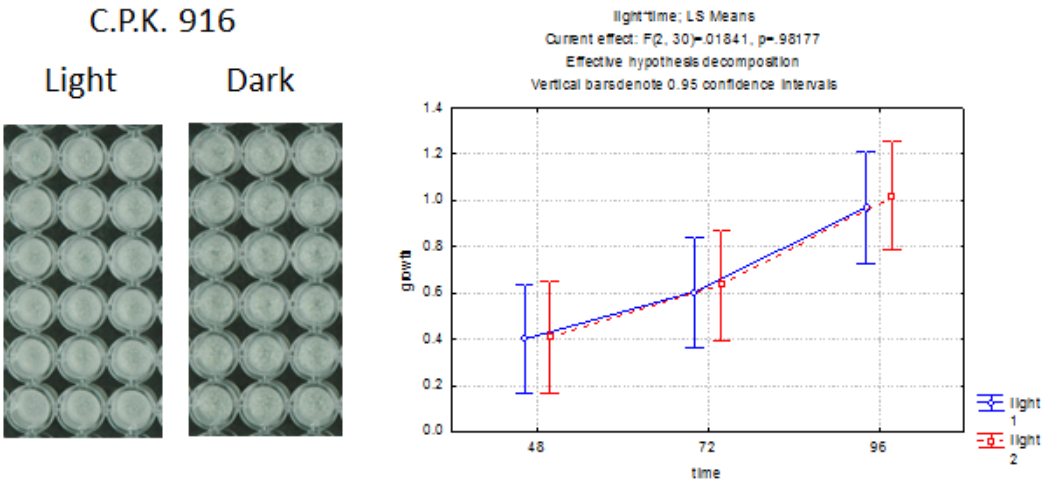

Figure 4.4.4. First part shows the sporulation of *T. harzianum* sensu stricto C.P.K. 916 after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

Three strains are presenting *T.* cf. *harzianum*. Two of them showed photoneutral results C.P.K. 295 (ANOVA, Current effect: F(2, 12)=6.3824, p=.01294) and C.P.K. 4455 (ANOVA, Current effect:

F(2, 30)=.56285, p=.57548), while the third strain C.P.K. 2389 (ANOVA, Current effect: F(2, 12)=25.666, p=.00005) have shown photostimulation. Photoneutral C.P.K. 295 has shown higher rates of conidiation under the dark conditions, photostimulated C.P.K. 2389 has shown higher conidiation under the blue light conditions and C.P.K. 4455 has shown no changes (Fig. 4.4.5)

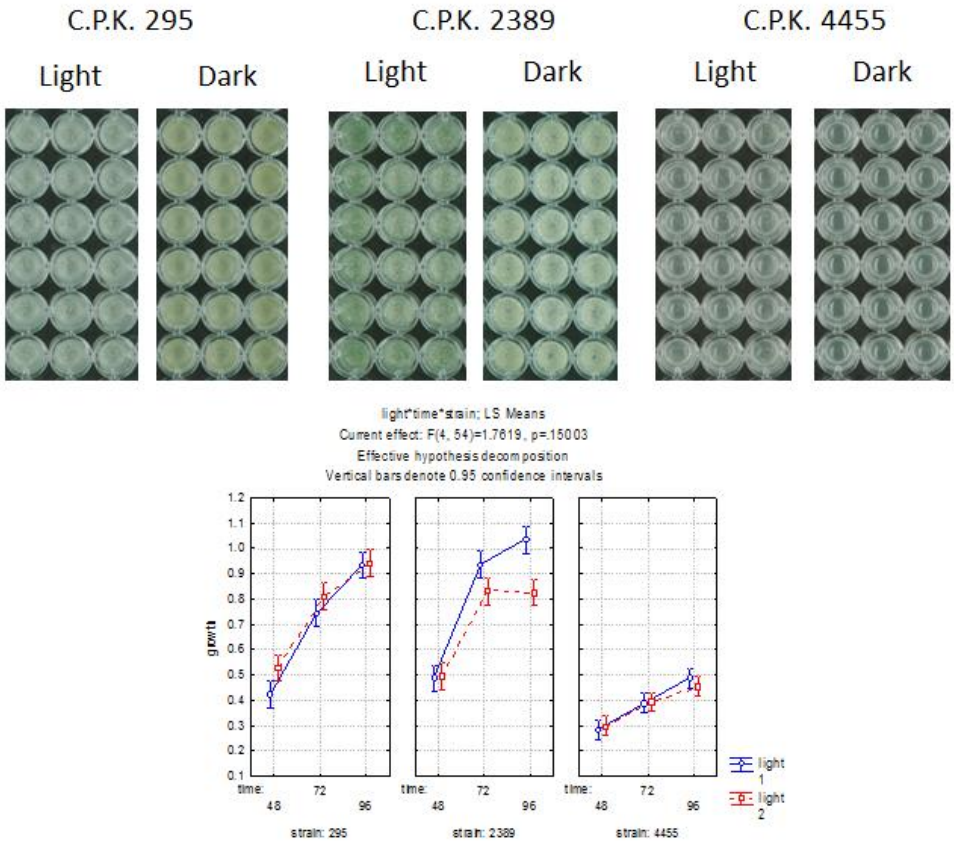

Figure 4.5.5. First part shows the sporulation of *T.* cf. *harzianum* strains after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

Two strains of *T. guizhouense* (*Harzianum* clade) have shown versatile growth responses to the illumination: while *T. guizhouense* C.P.K. 4742 strain was photoinhibited (Current effect: F(2, 12)=108.34, p=.00000), the *T. guizhouense* C.P.K. 4804 strain was largely photostimulated (Current effect: F(2, 12)=122.12, p=.00000). This pattern did not correspond to the conidiation that has been estimated at 72 hours of incubation. Both strains have shown higher levels of conidiation in light conditions (Fig. 4.4.6).

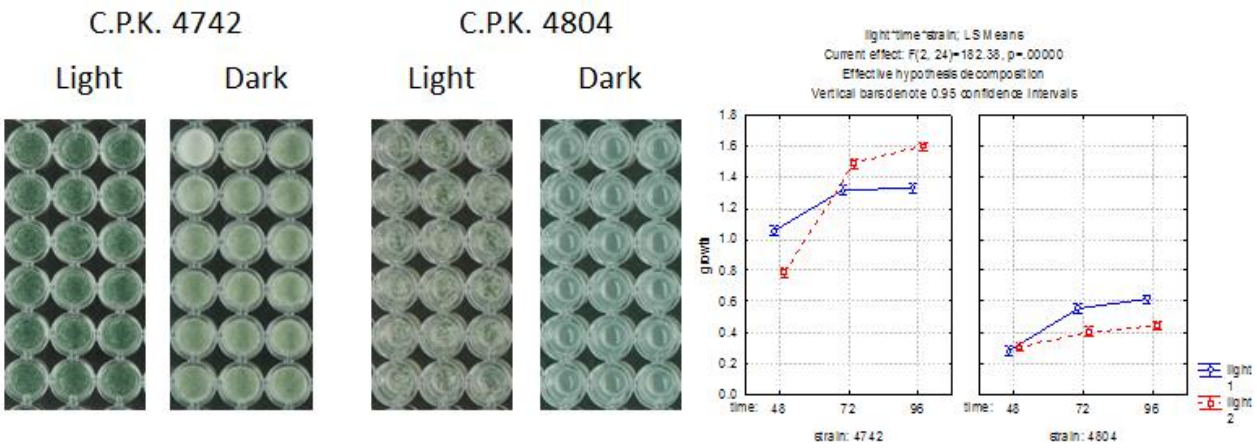

Figure 4.4.6. First part shows the sporulation of *T. guizhouense* strains after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

T. atroviride IMI 206040 (= C.P.K. 1680 in this study) is used as a model for photobiology of fungi. In our work six strains of *T. atroviride* have shown different results to the blue light stimulation: four strains C.P.K. 10 (Current effect: F(2, 12)=1.2381, p=.32446), C.P.K. 1680 (Current effect: F(2, 12)=56.092, p=.00000), C.P.K. 3020 (Current effect: F(2, 12)=3.0708, p=.08376) and C.P.K. 3027 (Current effect: F(2, 12)=107.66, p=.00000) were photostimulated, at the same time C.P.K. 2663 (Current effect: F(2, 12)=.08701, p=.91724) and C.P.K. 4498 (Current effect: F(2, 12)=.21418, p=.81022) were photoneutral. This data were confirmed with strains pictures on the plates (Fig. 4.4.7).

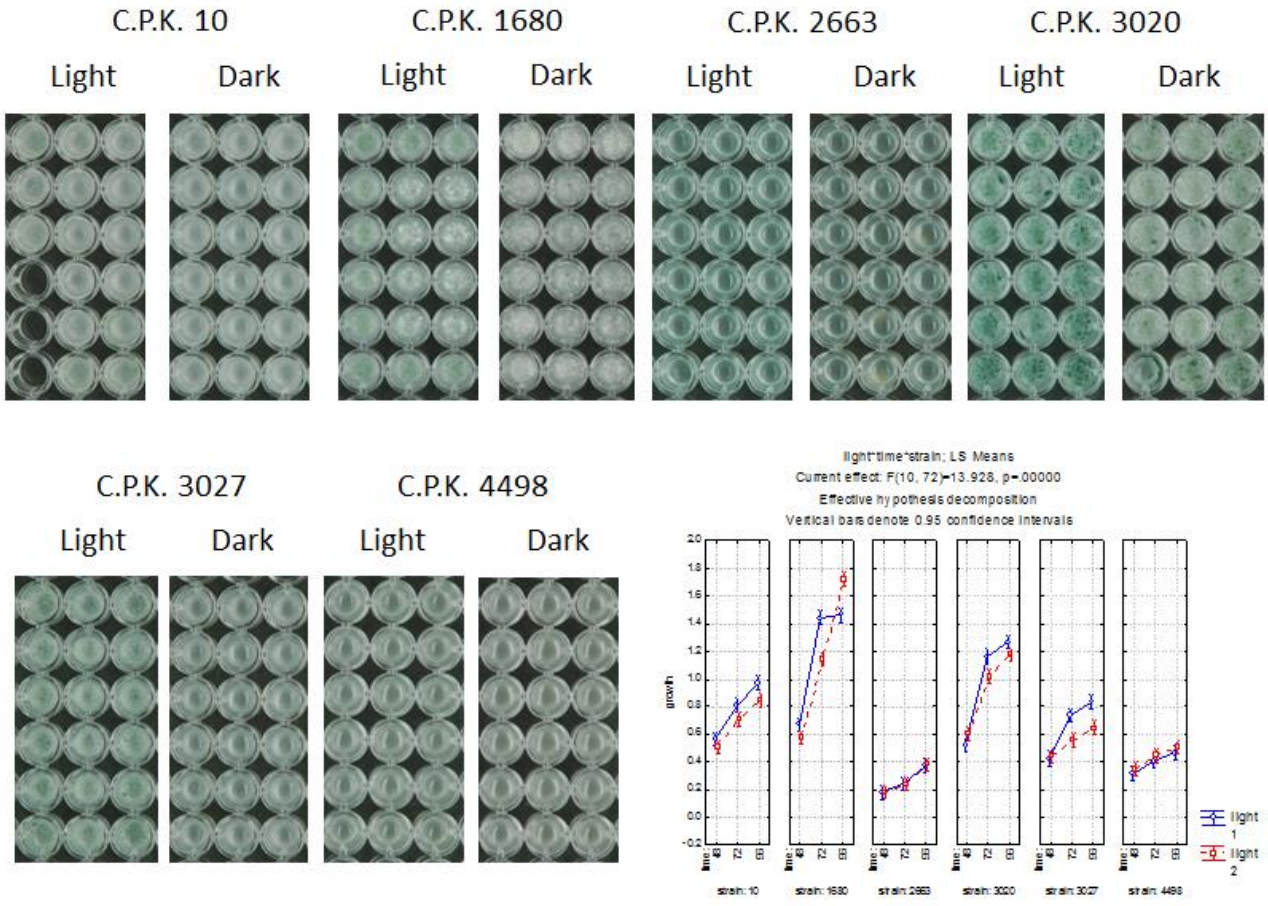

Figure 4.4.7. First part shows the sporulation of *T. atroviride* strains after 72 hours under blue light illumination and darkness. Second part shows the measurements of growth rates in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

4.5. Expression of blue light-dependent genes in selected *Trichoderma* spp.

To confirm differences in the expression of genes in *Trichoderma* genus under light and dark conditions three strains were selected: *T. parareesei* C.P.K. 717, *T. harzianum* C.P.K. 916 and *T. atroviride* C.P.K. 1680. Samples for RNA extraction were collected after 60 hours of the incubation (Fig. 4.5.1). Pictures of plates demonstrate that under the blue light illumination hyphal growth was stimulated and higher rates of conidiation were produced in compare to the dark conditions.

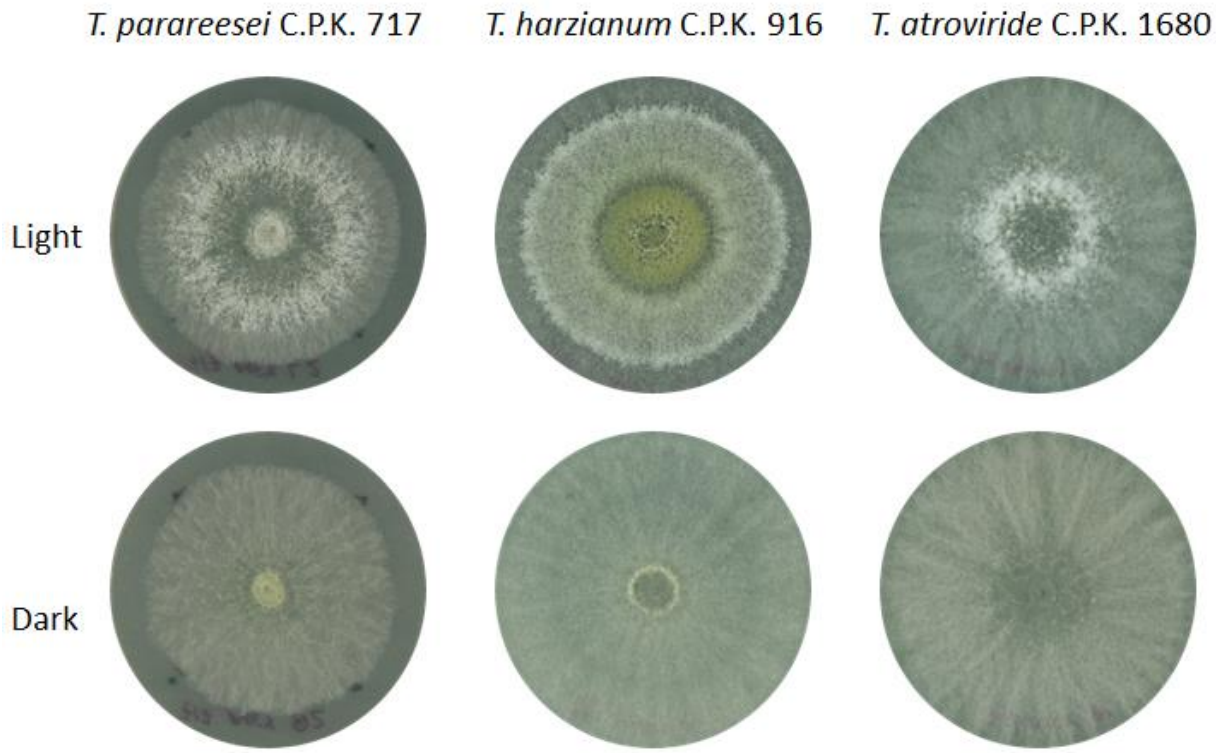

Figure 4.5.1. *T. parareesei* C.P.K. 717, *T. harzianum* C.P.K. 916 and *T. atroviride* C.P.K. 1680 after 60 hours of the incubation under blue light stimulation and dark conditions.

Concentrations of RNA obtained after RNA extraction as cDNA concentrations measured after cDNA synthesis are shown in the table below (Table 4.5.1).

№	C.P.K.	Light/Dark Conditions	RNA concentration ng/µl	cDNA concentration ng/µl
1	717	L	2019.5	2684.8
2	717	D	2013.9	2443.2
3	916	L	1701.0	2784.9
4	916	D	1432.8	2149.1
5	1680	L	1362.7	2573.5
6	1680	D	489.9	4532.6

Table 4.5.1. *T. parareesei* C.P.K. 717, *T. harzianum* C.P.K. 916 and *T. atroviride* C.P.K. 1680 original concentrations of RNA and cDNA.

cDNAs were diluted to the final concentration ranging from 45 to 60 ng/µl. Using specific primers designed for this experiment Reversed Transcription PCR (RT-PCR) was performed for 4 genes (*tef1*, *phr1*, *nox1* and *lae1*). Full information about primers and protocols used for this experiment is provided in supplementary materials (S3-S5) at the appendix part of this thesis.

Tef1 gene is a housekeeping gene of eukaryotic organisms including fungi. It was decided to use it as a control for the experiment. PCR products were loaded on agarose gel for electrophoresis. Results are presented on the pictures below (Fig. 4.5.2-4.5.4).

First picture (Fig. 4.5.2) is representing results for gene expression in *T. parareesei* under the different light conditions. As we can see *tef1* gene is expressed on a high level in both conditions (light and dark sample). *Phr1* and *nox1* genes have more readable bands in the samples collected under the dark growing conditions. Expression of *phr1* and *nox1* in *T. parareesei* in light conditions is on the low level and not clearly visible. Expression of *lae1*gene is on the same level in both (light and dark) samples and it is possible to say that it is not regulated by the blue light illumination.

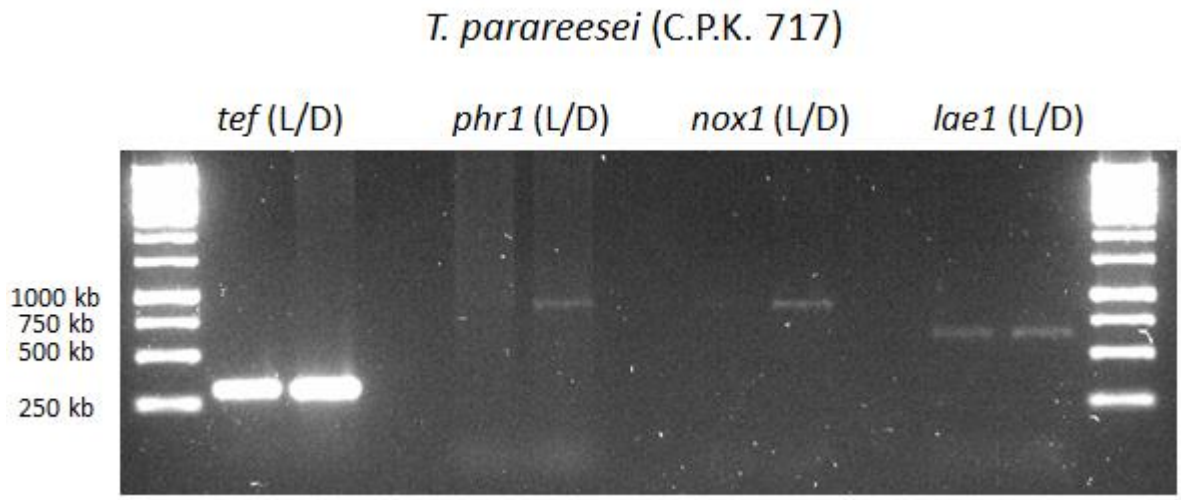

Figure 4.5.2. Gene expression in *T. parareesei* under the light (L) and dark (D) conditions.

Next picture (Fig. 4.5.3) involves results for gene expression in *T. harzianum* under the different light conditions. Situation here is completely different. There is no visible expression of *phr1* and *lae1* genes. Per contra *nox1* bands are showing higher expression levels in dark conditions although it is presented in the sample collected under the light conditions too.

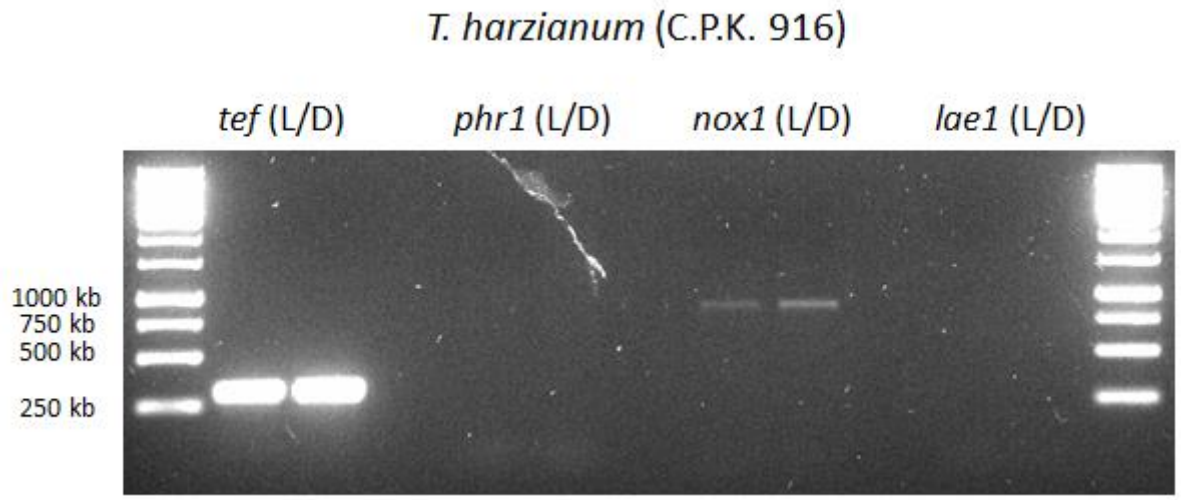

Figure 4.5.3. Gene expression in *T. harzianum* under the light (L) and dark (D) conditions.

The last picture (Fig. 4.5.4) shows results for gene expression in *T. atroviride* under the different light conditions. *Phr1* is expressed on very low level in both conditions. *Nox1* is not expressed in *T. atroviride* samples and not visualized on the picture. *Lae1* gene shows higher expression level in dark conditions.

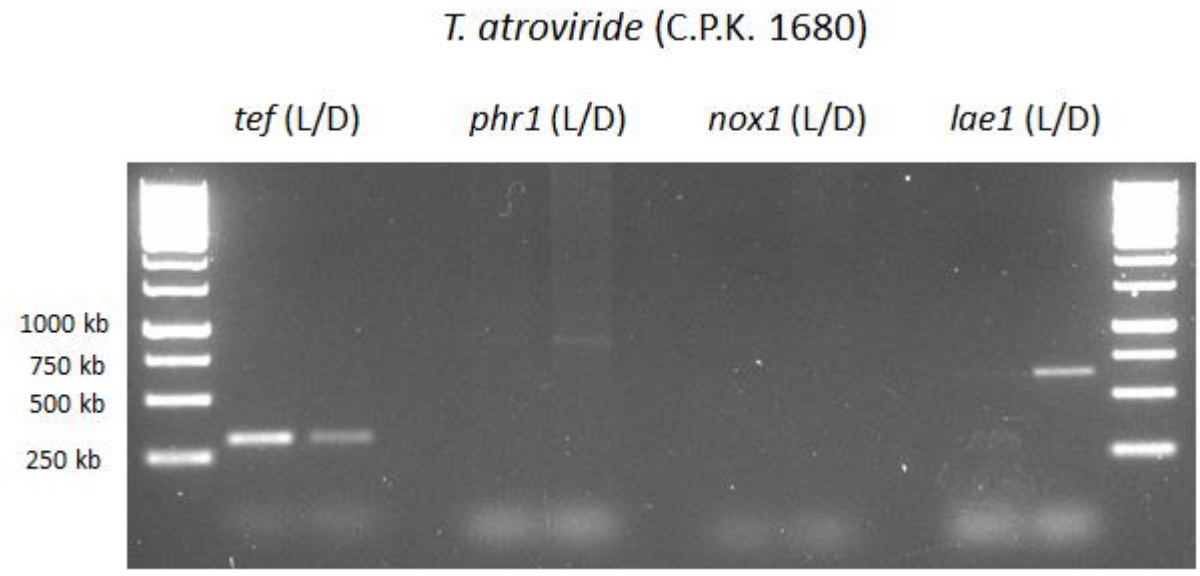

Figure 4.3.4. Gene expression in *T. atroviride* under the light (L) and dark (D) conditions.

As we can see all genes that were tested in this experiment are showing various results in different *Trichoderma* species. However it is possible to propose that *phr1*, *nox1* and *lae1* are showing higher expression rates under the dark conditions in compare to the light conditions of the cultivation.

5. Discussion

The results of this master thesis are experimental observations of the photoresponse of *Trichoderma* in a genus-wide spectrum. The highly effective method for the assessment of fungal response to the blue light illumination has been developed. In the course of the experiment 123 strains of *Trichoderma* genus (25 of them were represented by mutants) were used.

The results of the experiment have demonstrated that *Trichoderma* genus in general is photoinsesitive to the blue light spectrum. Phylogenetically related species of *Trichoderma* genus have demonstrated versatile response to the blue light photostimulation (450 nm). Moreover even stains that represent the same species can be photoinhibited and photostimulated at the same time. It can be supposed that different photosensitivity of each strain related to attempts to produce more successful strategy for reproduction, dispersal and general survival in different environmental conditions.

Data analysis showed that endophytic strains *T. atroviride* C.P.K. 2663, *T. koningiopsis* C.P.K. 4884, *T. orientalis* C.P.K. 109, *T. harzianum* s.1. (endophyticum) C.P.K. 4893, *T. harzianum* s.1. (neurotropicale) C.P.K. 4896 and *T. spirale* C.P.K. 4896 isolated from plants have shown positive photostimulation. It is possible to speculate that such behavior of strains is stimulated with the opportunity to occupy more convenient ecological niche (for example leaves and not stems of the plants). Another hypothesis could be explained by the cyclical intensity of the blue light in the natural conditions of temperate zone. It is observed that the blue light spectrum reaches its maximum intensity in the autumn period. At the same time plants are starting the defoliation process. In this situation fungi strain will be stimulated by the blue light in a habitat full of decaying plant rests in the soil. Such conditions could be an advantage for the successful competition with other organisms.

In course of the experiment the groups of *Trichoderma* genus, represented by 2 or more strains with the same response to photostimulation, were described. Interestingly that in the course of data analysis were discovered no examples of species represented by several strains that would be photostimulated or photoneutral. The only photoinhibited species or species with versatile results were observed.

Despite the fact that results obtained from *Aspergillus* and *Escovopsis* genera showed photoinsencitivity to the blue light spectrum, experiments in this direction should be continued. Further studies, for which no time was available during this work, can analyze much more species from these two genera using the developed method to obtain the comprehensive and full data.

The findings of this master thesis confirm the findings obtained earlier by Friedl et al. 2008 and Herrera-Estrella 2012 and certify that photostimulation leads to hyphal growth and increased level of conidiation in *T. atroviride*. However this species seems to be rather exceptional in the genus.

Moreover by *T. virens* example it was confirmed the findings of Gupta and Ayyachamy 2012 and demonstrated that the knocked out *Δvel1* gene leads to the higher level of photoinhibition by mutants in comparison with wild strains.

The experimental data showed that *xyr1* mutants did not show any significant effect linked to the blue light stimulation which also requires more detailed researches in this area.

In the course of data analysis of various strains conidiation it was observed that strain *T.* cf. *harzianum* (C.P.K. 295) showed increased level of conidiation in the dark conditions, that does not fit into the overall picture describing other strains of this species.

In the second part of the master thesis the specific primers have been designed and the expression of three genes involved in the photo regulation (*phr1, nox1* and *lae1*) in *T. parareesei*,

T. harzianum and *T. atroviride* in light and dark conditions has been shown. Based on the results of this experiment we can conclude that these photo-regulated genes display a higher expression level in the dark.

It was reported earlier that in the visible region the light effectiveness for photoinduction extends to 550nm, while in photorepair it extends only to about 430nm (Gloria M. Berrocal-Tito et al. 2007). On the basis of these facts it is possible to suggest that the high expression of *phr1* in *T. atroviride* requires the stimulation by the light source with the different length of light wave.

All the tasks set for this work have been successfully completed. We investigated the response of *Trichoderma* genus to illumination on the broad generic level. The semi-hight throughput method for the assessment of fungal response to illumination has been developed. The screening of the genus *Trichoderma* covering all major infrageneric phylogenetic clades has been performed. The comparison with some other filamentous Ascomycota has been provided.

The data collected in the master thesis and related to the photostimulation of *Trichoderma* genus are based on a huge amount of researched material as a whole. Thus they certainly can be used for further researches in this field.

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8. Lebenslauf

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Sprachkenntnisse

Ukrainisch, Russisch, Englisch, Deutsch, Schwedisch (A1).

Besondere Kenntnisse

Office-Programme: Word, Excel, PowerPoint.

Führerschein Klasse B.

9. Appendix

- **S1 - Reverse transcription protocol**

1. Protocol for Preparation of DNA-free RNA prior to RT-PCR- Promega DNAse

a. Add to a RNAse-free tube: Don't vortex the RNA(middle or no shaking on termoblok!)

RNA	1µg
10x reaction buffer with MgCl2	1 µl
DEPC-treated Water	to 9 µl
Deoxyribonuclease I (DNase I),RNase free (1u/ µl)	1 µl(u)

Note: scale up if larger amounts of DNA-free RNA are required!

b. Incubate at 37°C for 30 min

c. Add 1 µl 25mM EDTA and incubate at 65°C for 10 min.

RNA hydrolyses during heating in the absence of a chelating agent (d).

d. Use the prepared RNA a template for reverse transcriptase.- >put on ice.

2. Fermentas Kit: work on ice!

a. Prepare following reaction mixture in RNase free tube on ice:

i. Total RNA 10ng-5 µg= 11 µl

ii. Oligo (dT)18 primer(0.5 µg/µl) 1 µl+1 µl=12 µl

iii. DEPC-treated water to12 µl

Mix gently and centrifuge briefly!

b. Incubate the mixture at 70°C for 5 min, chill on ice and centrifuge briefly!

c. Place the tube on ice and add the following components:

i. 5x reaction buffer 4 µl

ii. RiboLock Ribonuclease inhibitor (20u/µl) 1 µl

iii.10mM dNTP mix 2 µl

d. Incubate at 37°C for 5 min

e. Add RevertAid H Minus M-MulV RT(200u/µl) 1µl

Final volume 20µl

f. Incubate the mixture at 42°C for 60 min.

g. Stop the reaction by heating at 70°C for 10 min. Chill on ice.

e. Freeze samples in -80ºC or in -20ºC for short time.

- **S2 - PCR Protocol for control of designed primers and T_m optimization with gDNA.**

	stock in M	working M	end M	µl (1)	Mix
sample				5.00	50
Promega buffer 5 -fach	10	1	1	5.00	50
Promega Nucleotide 10mM	0.01	0.002	1.60E-04	4.00	40
MgCl2 25mM	0.025		3.00E-03	6.00	60
1-fw 100µM	0.0001	6.25E-06	2.50E-07	2.00	20
1- rev 100µM	0.0001	6.25E-06	2.50E-07	2.00	20
Promega Taq U/µl	5		0.5	0.10	1
water				25.90	259
			Объем	50.00	
			Объем смеси		450
			premix volume to pipett		45.0
			проверка=	50.00	45
Количество проб= (включая "-" контроль)	10				

PCR

bigmachine	prog 38	
	min	temp C
initial denaturation	2	95
30 cycles	45	95
	1	gradient
	3	72
final extension	10	72
pause		14

IMAGE

phoresis

agarose	0.015
bp ladder	+
dark blue dye	+
product	10µl
	80 V
30 min	200 mA

track	Sample	Product	
1		-	
2	neg control water	-	water
3	a		51.5
4	b		53.1
5	c		54.6
6	d		56.2
7	e		57.8
8	f		59.4
9	g		60.9
10	h		62.5
11			
12			
13			
14			
15			
16			

- **S3 - PCR Protocol for “PHR1 and LAE1 expression”**

	stock in M	working M	end M	µl (1)	Mix
sample				5.00	45
Promega buffer 5 -fach	10	1	1	5.00	45
Promega Nucleotide 10mM	0.01	0.002	1.60E-04	4.00	36
MgCl2 25mM	0.025		3.00E-03	6.00	54
1-fw 100µM	0.0001	6.25E-06	2.50E-07	2.00	18
1- rev 100µM	0.0001	6.25E-06	2.50E-07	2.00	18
Promega Taq U/µl	5		0.5	0.10	0.9
water				25.90	233.1
			Объем	50.00	
			Объем смеси		405
			premix volume to pipett		45.0
			проверка=	50.00	45
Количество проб=	9				
(включая "-" контроль)					

PCR

bigmachine	prog 38	
	min	temp C
initial denaturation	2	95
30 cycles	45	95
	1	63
	3	72
final extension	10	72
pause		14

IMAGE

5 MM

phoresis

agarose	0.015
bp ladder	+
dark blue dye	+
product	10µl
	80 V
30 min	200 mA

- **S4 - PCR Protocol for "NOX1 and TEF1 expression"**

	stock in M	working M	end M	µl (1)	Mix
sample				5.00	45
Promega buffer 5 -fach	10	1	1	5.00	45
Promega Nucleotide 10mM	0.01	0.002	1.60E-04	4.00	36
MgCl2 25mM	0.025		3.00E-03	6.00	54
1-fw 100µM	0.0001	6.25E-06	2.50E-07	2.00	18
1- rev 100µM	0.0001	6.25E-06	2.50E-07	2.00	18
Promega Taq U/µl	5		0.5	0.10	0.9
water				25.90	233.1
			Объем	50.00	
			Объем смеси		405
			premix volume to pipett		45.0
			проверка=	50.00	45
Количество проб=	9				
(включая "-" контроль)					

PCR

bigmachine	prog 38	
	min	temp C
initial denaturation	2	95
30 cycles	45	95
	1	56
	3	72
final extension	10	72
pause		14

IMAGE

5 MM

phoresis

agarose	0.015
bp ladder	+
dark blue dye	+
product	10µl
	80 V
30 min	200 mA

- **S5 - List of designed primers.**

***PHR1* primers for all strains**

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GACTTTGAGGCTCATCTCAC	Plus	20	532	551	55.87	50.00	7.00	1.00
Reverse primer	GCAGCGTCGACAATGGG	Minus	17	1457	1441	58.81	64.71	6.00	2.00
Product length	926								

***NOX1* primers for all strains**

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GATGAGAATCTGTGGATGC	Plus	19	345	363	53.23	47.37	5.00	2.00
Reverse primer	GTTTCGGAGATGCCAGATG	Minus	19	1253	1235	56.09	52.63	3.00	2.00
Product length	909								

LAE1* primers for *T. parareesei* and *T. harzianum

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGCTTTTATGGTTCTTGGAAGC	Plus	22	43	64	58.50	45.45	4.00	3.00
Reverse primer	GACCACGGGTTTACGTAGCA	Minus	20	545	526	60.04	55.00	6.00	2.00
Product length	503								

LAE1* primers for *T. atroviride

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CGATTCTATGGCAACTGGAAGA	Plus	22	175	196	58.20	45.45	4.00	0.00
Reverse primer	GACCACGGGTTCAAATAGCA	Minus	20	824	805	58.18	50.00	4.00	1.00
Product length	650								

- **S6 - Measurements of growth rates of photoinhibited species.**

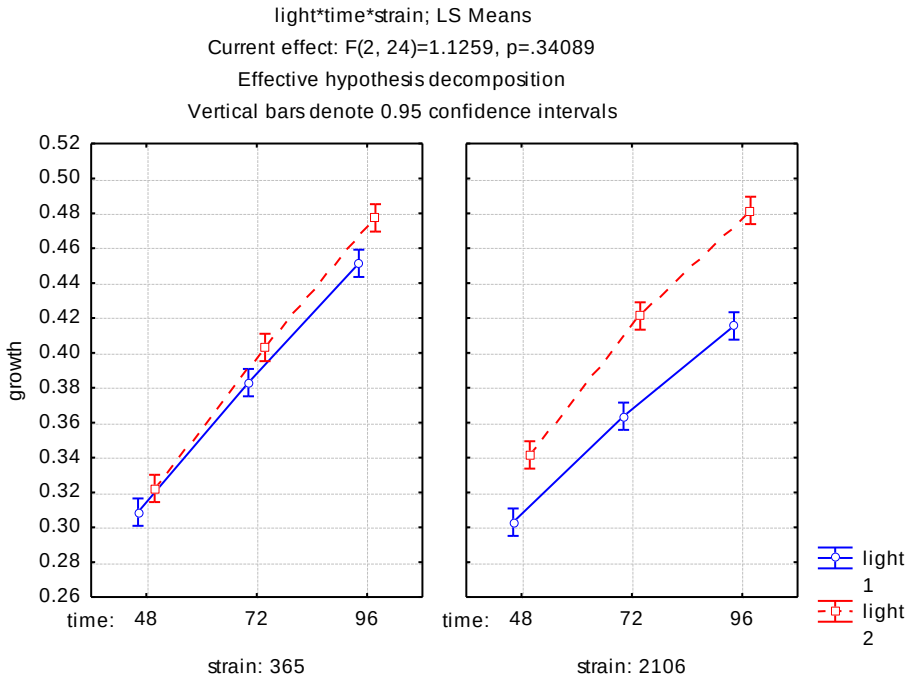

Figure S6. The measurements of growth rates of *T. aggressivum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

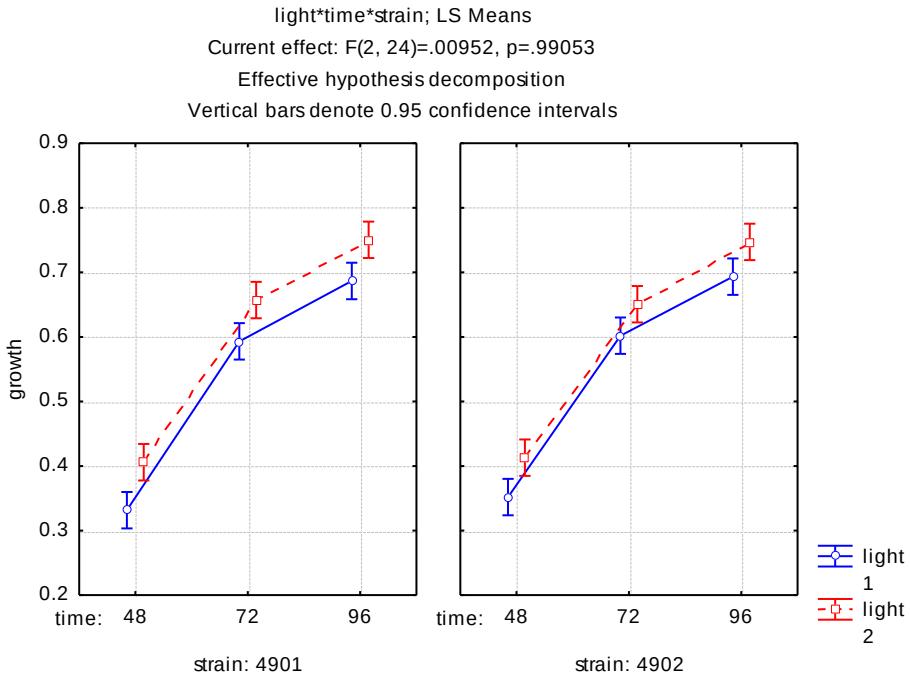

Figure S6 The measurements of growth rates of *T. amazonicum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

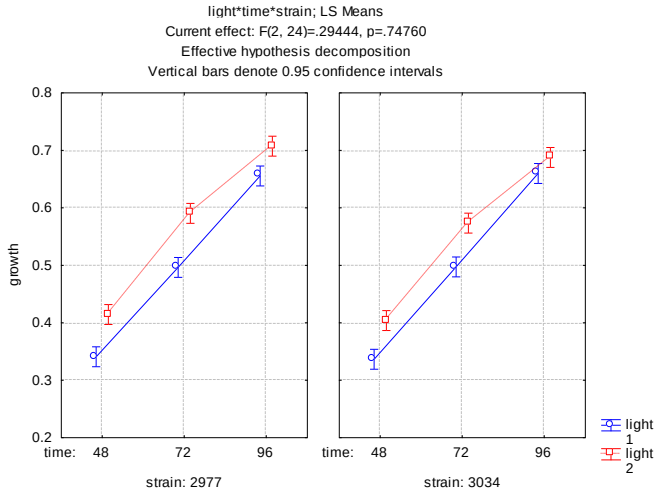

Figure S6 The measurements of growth rates of *T. tomentosum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

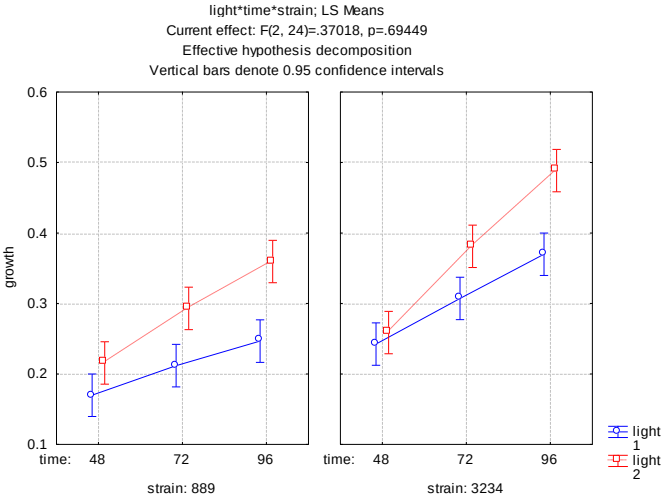

Figure S6 The measurements of growth rates of *T. rossicum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

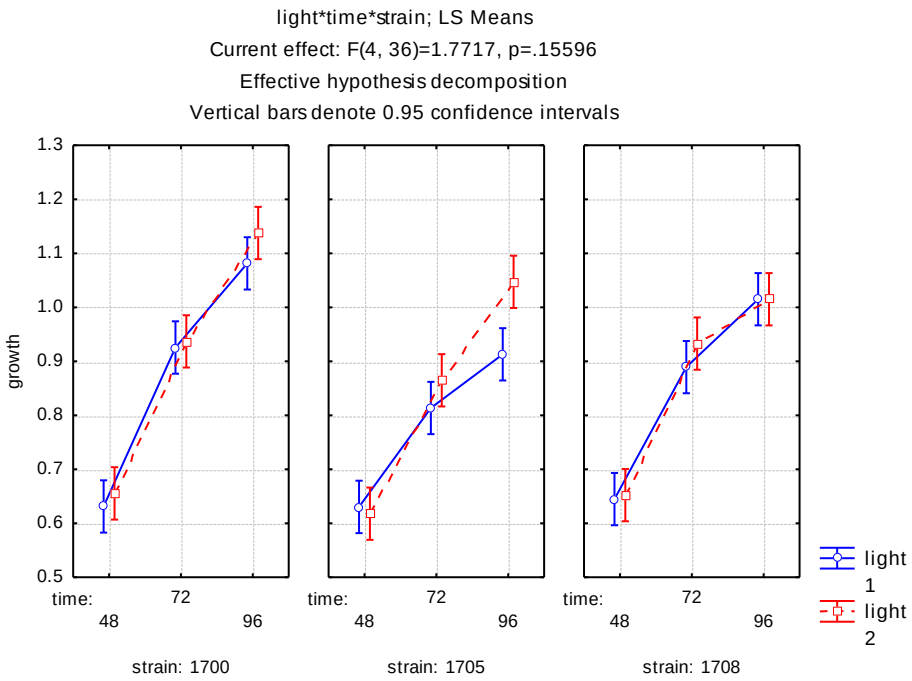

Figure S6 The measurements of growth rates of *T. citrinoviride* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

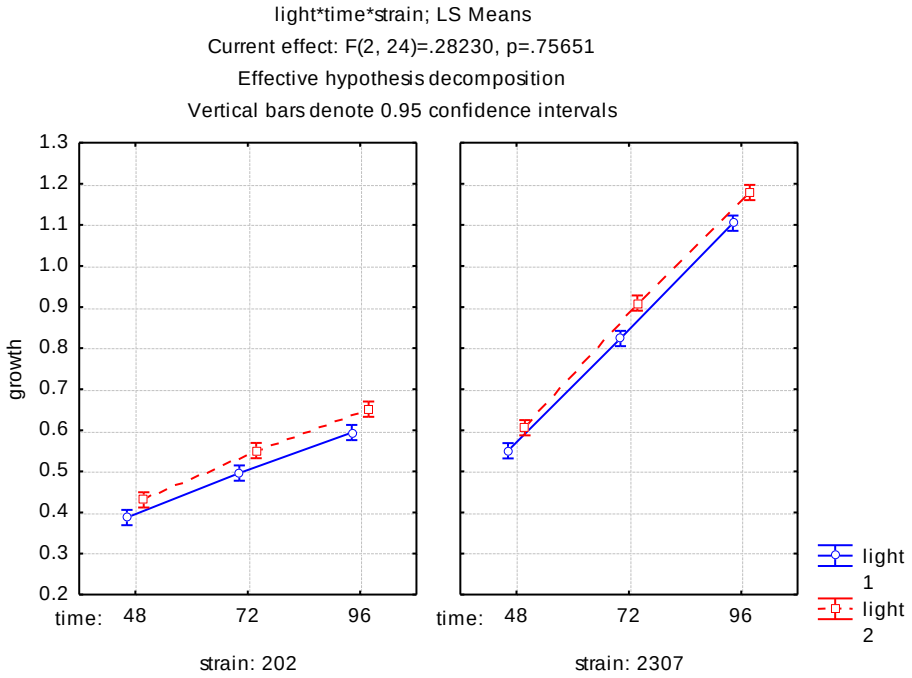

Figure S6 The measurements of growth rates of *T. hamatum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

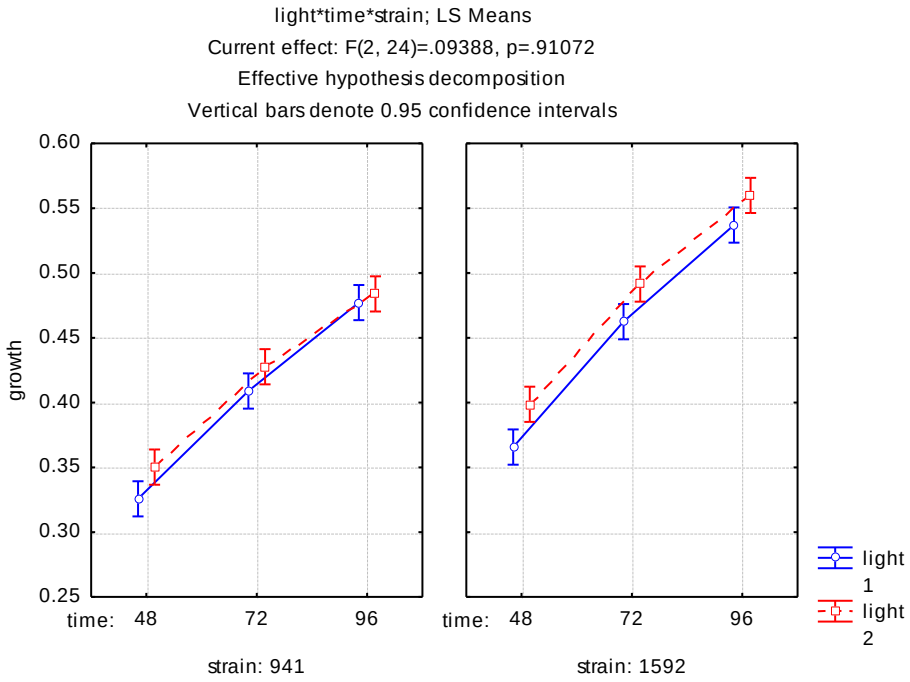

Figure S6 The measurements of growth rates of *T. voglmayrii* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

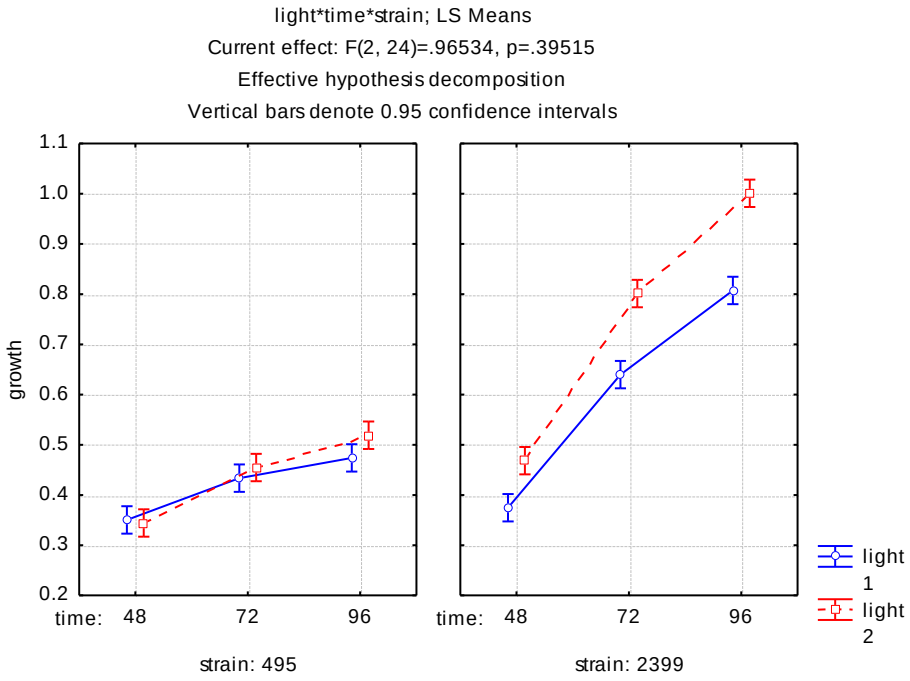

Figure S6 The measurements of growth rates of *T. minutisporum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

- **S7 - Measurements of growth rates of versatile species.**

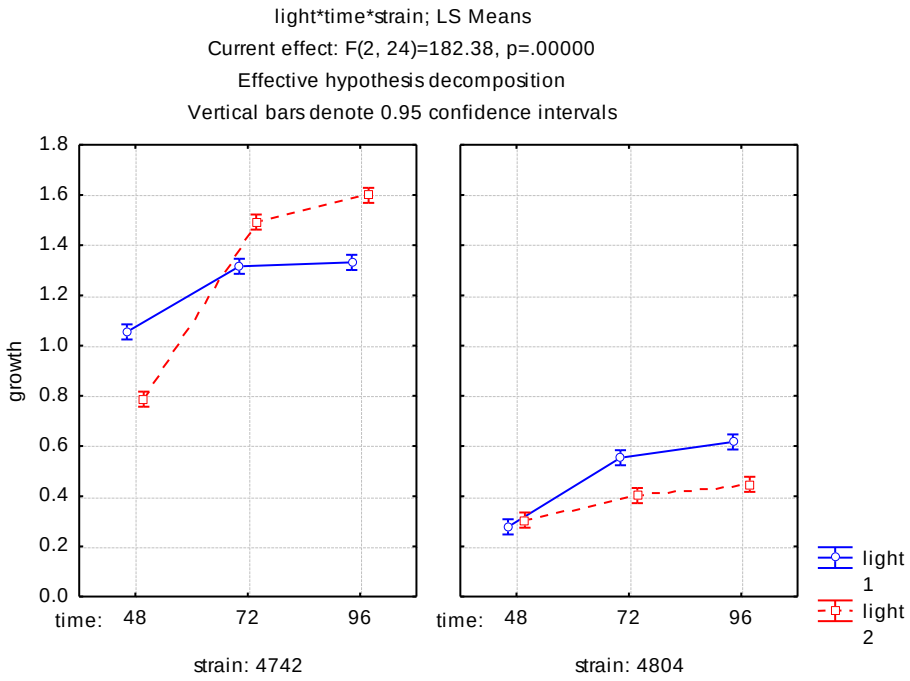

Figure S7 The measurements of growth rates of *T. gouizhouense* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

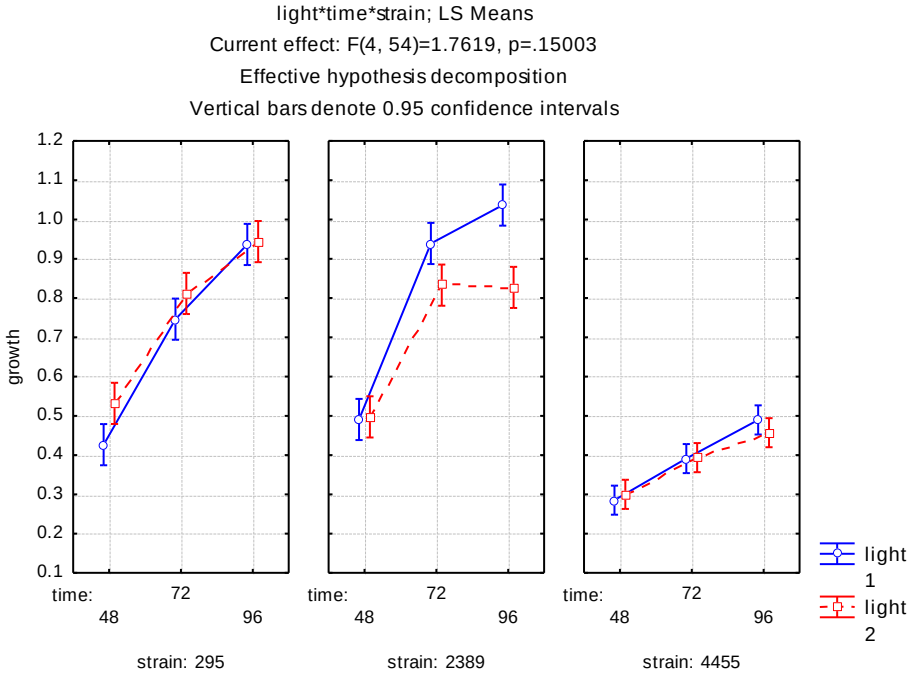

Figure S7 The measurements of growth rates of *T.* cf. *harzianum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

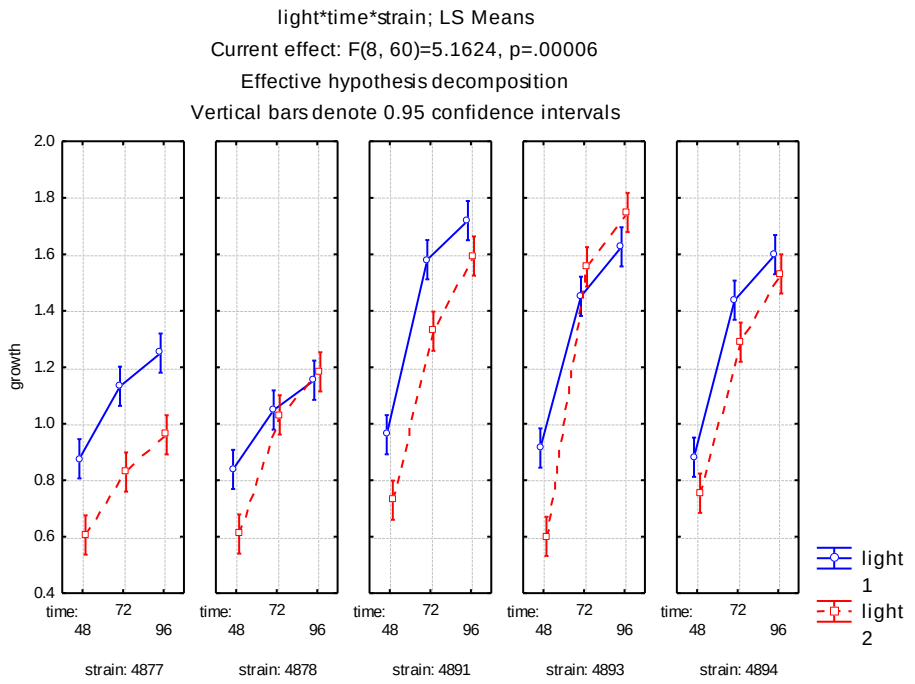

Figure S7 The measurements of growth rates of *T. harzianum* s.I. (Endophyticum) in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

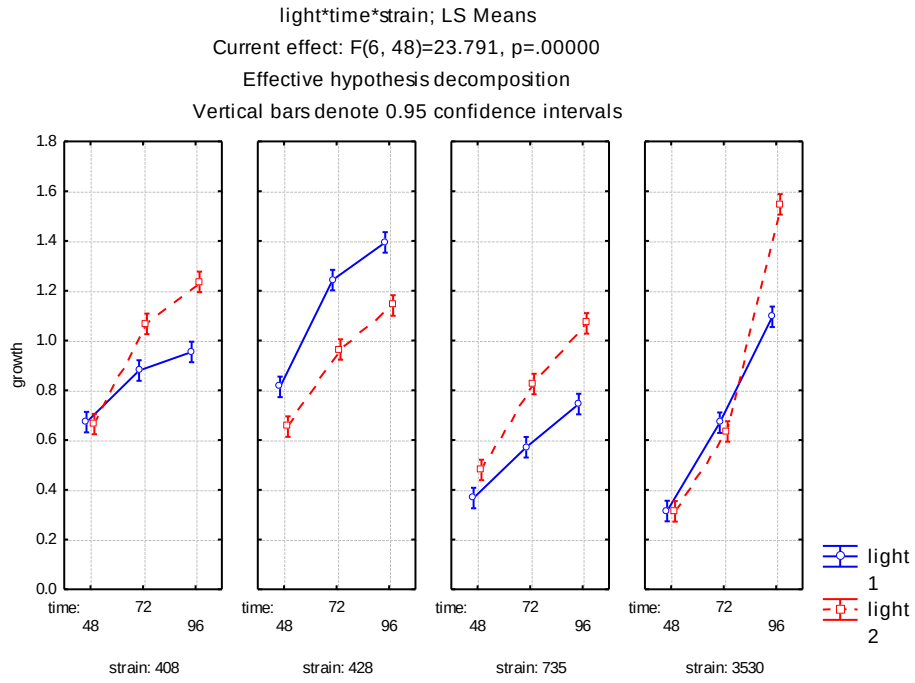

Figure S7 The measurements of growth rates of *T. virens* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

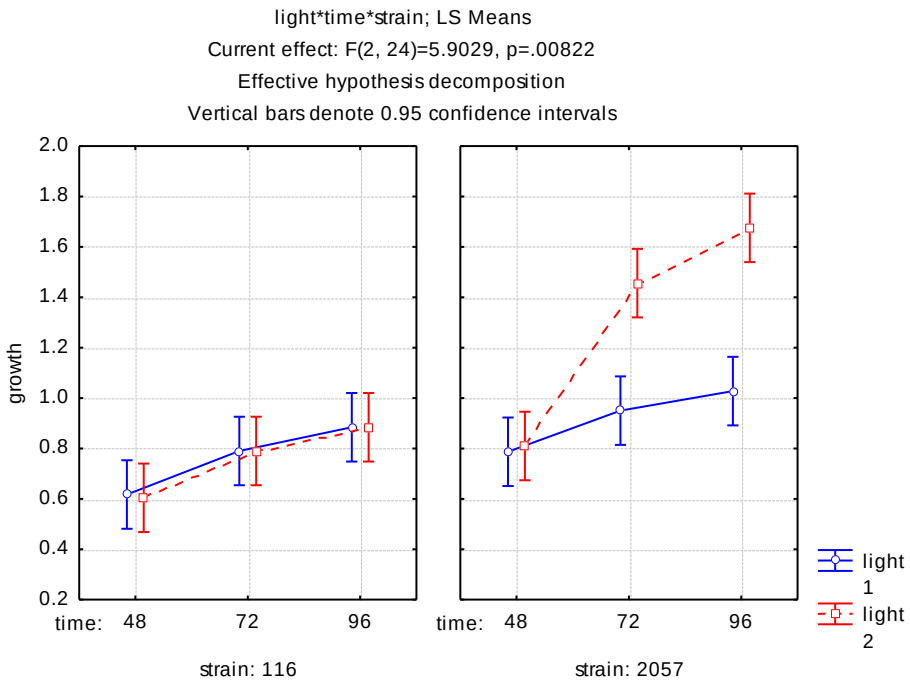

Figure S7 The measurements of growth rates of *T. ghanense* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

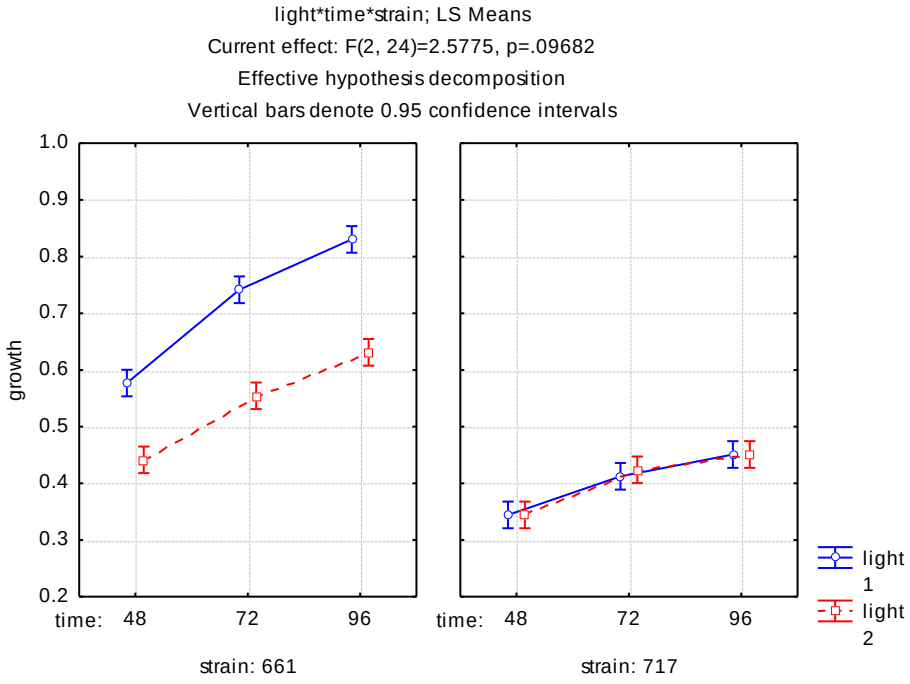

Figure S7 The measurements of growth rates of *T. parareesei* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

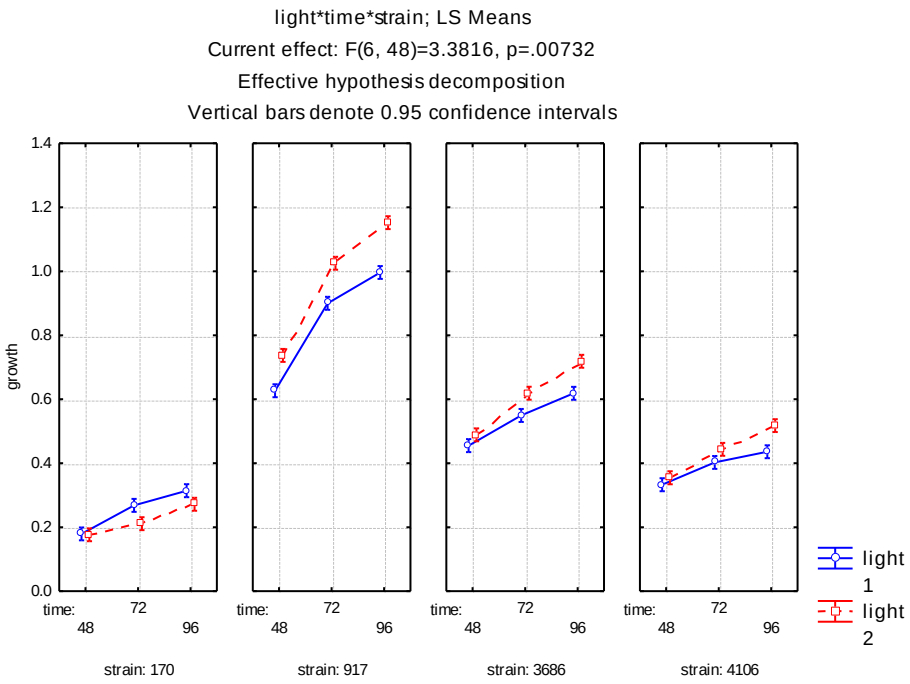

Figure S7 The measurements of growth rates of *T. reesei* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

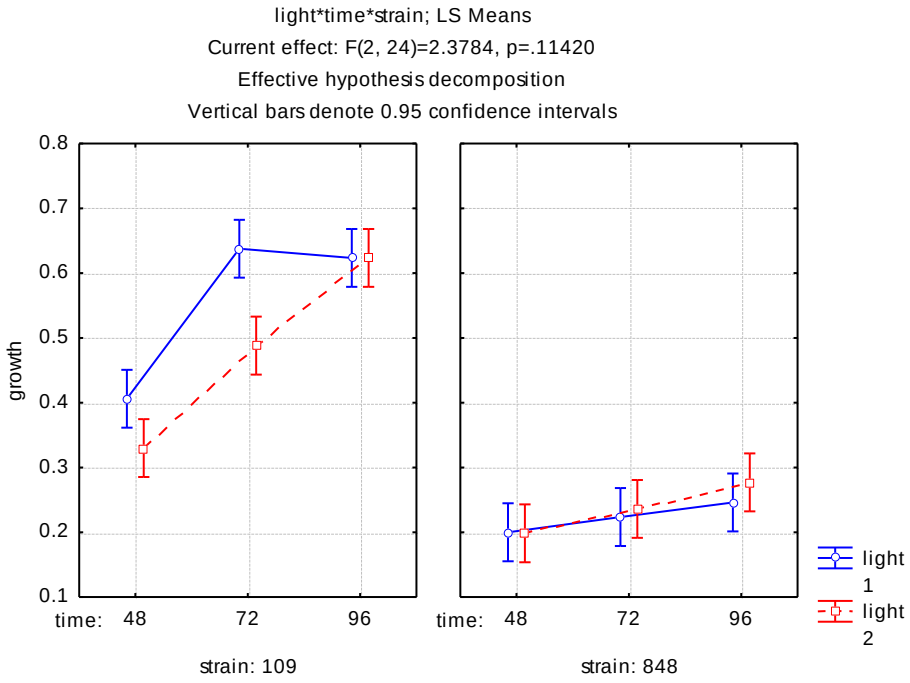

Figure S7 The measurements of growth rates of *T. orientalis* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

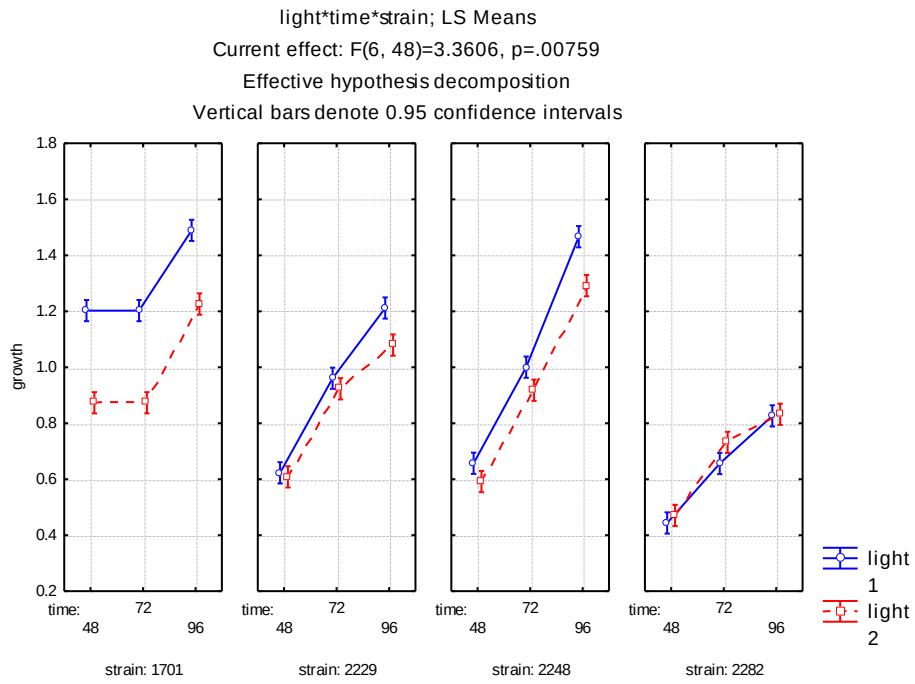

Figure S7 The measurements of growth rates of *T. longibrachiatum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

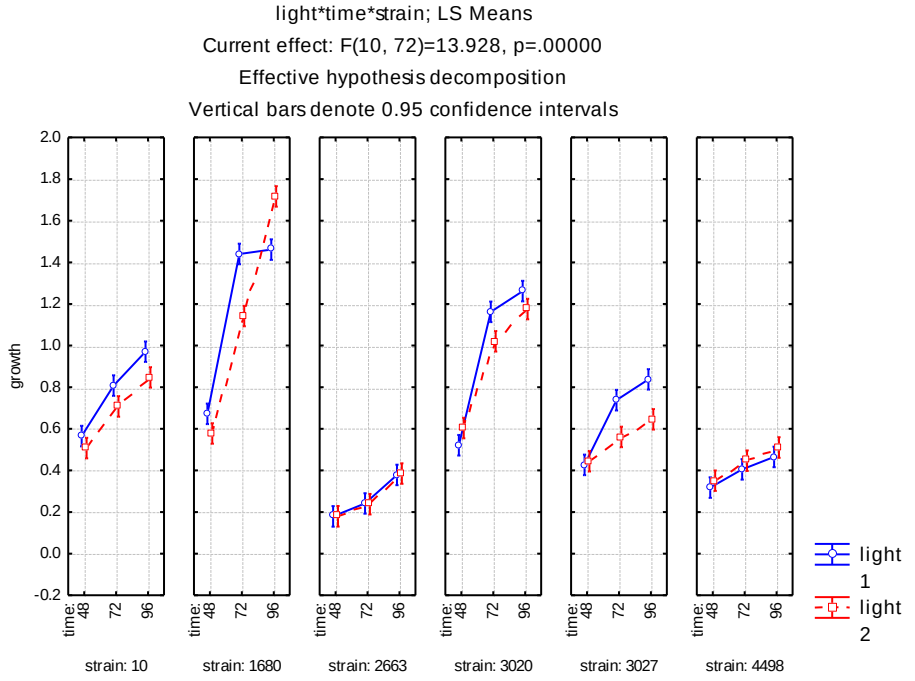

Figure S7 The measurements of growth rates of *T. atroviride* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

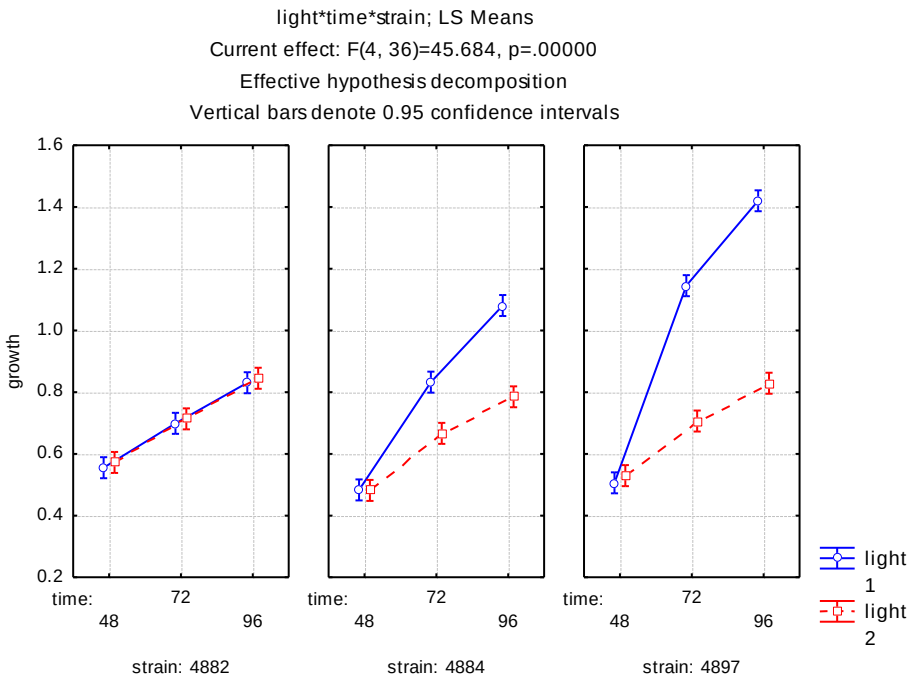

Figure S7 The measurements of growth rates of *T. koningiopsis* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

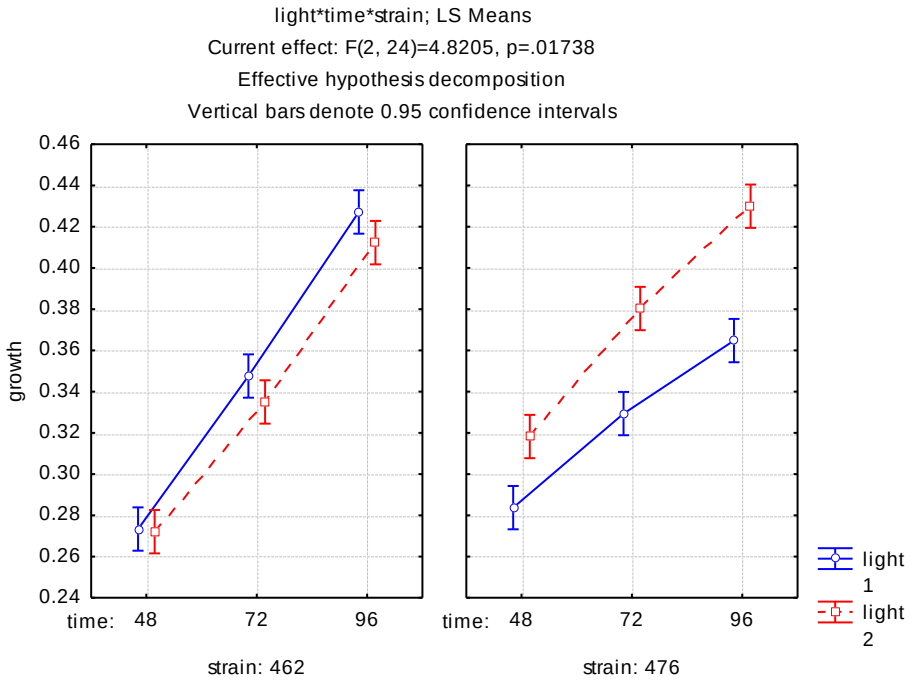

Figure S7 The measurements of growth rates of *T. polysporum* in dark (red line) and light (blue line) conditions after 48, 72 and 96 hours.

- **S8 – Bootstrap values obtained from the maximum parsimony analysis based on 500 replicates (Figure 4.1.2).**