Diplomarbeit

Antimicrobial activity of the rare and novel fungi isolated from the high canopy of Borneo rain forest

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Abstract

Secondary metabolites (SM) are the small organic bioactive molecules that are produced by microorganisms or plants for the variety of ecological functions that are not linked to the primary metabolism. Thus, SM are used for defence against biotic (all forms of symbiosis) or abiotic factors, communication or parasitic attacks. Probably, the best known SMs of fungi and bacteria are antibiotics that were discovered in the mid-20th century to treat the diversity of bacterial diseases. However, in the last decades, a lot of pathogens, especially bacteria, achieved a resistance against the commonly used antibiotics. The antimicrobial resistance occurs naturally, by horizontal gene transfer, but it is accelerating by the mis and overuse of antibiotics worldwide. Consequently, the new antibiotics and antibiotic producers need to be discovered.

In this thesis, we aimed to set up a pipeline to test the antimicrobial activity of novel and rare fungi, isolated from the high canopy of Borneo rainforest. For this purpose, we used the library of 40 fungal strains, that was enriched in putatively new taxa with unknown properties, but also several model producers of SMs with antimicrobial activity. To find the best antimicrobial SM (ASM) producer and the best inducing conditions, we screened several nutritional media (PDA, Dox, Malt Ex, PYG, YES, YESD, YPSS, Rice, Oat Flour, YM) and tested the ability of fungi to produce ASM under stress conditions (co-cultures and starvation). We also tested the antimicrobial activity of SM extracts (methanol/dichloromethane) of selected fungi against four non-pathogenic model organisms: *Escherichia coli*, *Bacillus velezensis*, *Saccharomyces cerevisiae* and *Fusarium oxysporum*. The antimicrobial activity of tested samples against *E. coli*, *B. velezensis* and *S. cerevisiae* could be checked visually after one day at 28°C by an appearing inhibition zone (halo) around the plugs. For *F. oxysporum* a cultivation time of three days at 28°C was necessary.

The results showed, that media composition has an influence on the ASM production of fungi. Furthermore, cultivation time plays a key role in the production of ASMs. With co-cultures, an adaptation of inhibitory compounds could be achieved with several strains. A few SMs extracts showed the minimum inhibitory concentration (MIC) against *E. coli*, *B. velezensis* and *S. cerevisiae*, but no ASM extracts were active against *F. oxysporum*.

We have selected the four best ASM producer strains, - *Penicillium expansum* (TUCIM 5626), *Ovicillium* sp. (TUCIM 5628), *Xylaria* sp. (TUCIM 5712) and *Arthrinium rasikravindrii* (TUCIM 5773) and suggest them for the further exploration by the NGS and -omics technologies.

Kurzfassung

Sekundärmetabolite (SM) sind kleine organische Moleküle, die von Mikroorganismen oder Pflanzen gebildet werden, um eine Vielzahl an ökologischen Funktionen, die nicht dem Grundstoffwechsel des Organismus zugeschrieben werden können, zu übernehmen. Diese Funktionen liegen unter Anderem in der Abwehr von biotischen- (jede Art von Symbiose), abiotischen Faktoren und Parasiten, sowie in der Kommunikation. Die wohl bekanntesten SM, die von Pilzen und Bakterien produziert werden, sind Antibiotika. Diese wurden hauptsächlich Mitte des 20 Jahrhunderts entdeckt und seitdem zur Behandlung von bakteriellen Krankheiten eingesetzt. Trotz der Verwendung von zahlreichen unterschiedlichen Antibiotika, zeigen immer mehr Erreger, speziell Bakterien, eine Resistenz gegen diese Medikamente. Diese Resistenz entsteht auf natürlichem Weg, durch horizontalen Gen-Transfer, jedoch führt übermäßiger und falscher Gebrauch von Antibiotika zu einer Beschleunigung dieses Phänomens.

In dieser Arbeit wollten wir einen Leitfaden zum Testen der antimikrobiellen Aktivität von neuen und seltenen Pilzen, die aus dem Regenwald von Borneo isoliert wurden, aufstellen. Dafür wurden 40 Pilzstämme verwendet, die aus potentiell neuen Arten mit bislang unbekannten Eigenschaften, sowie bereits etablierte SM Produzenten bestanden haben. Um den besten antimikrobiellen (ASM) Produzenten und die optimalsten Kultivierungsbedingungen zu finden, wurden unterschiedliche Medienzusammensetzungen (PDA, Dox, Malt Ex, PYG, YES, YESD, YPSS, Rice, Oat Flour, YM) und die Fähigkeit, SM unter Stressbedingungen (Co-Kulturen und Limitierung der Ressourcen) zu produzieren, getestet. Weiters wurden die gebildeten SM mit Hilfe von Methanol/Dichlormethan extrahiert und die antimikrobielle Aktivität gegen vier nicht-pathogene Organismen (*Escherichia coli, Bacillus velezensis*, *Saccharomyces cerevisiae* und *Fusarium oxysporum)*, getestet. Die antimikrobielle Aktivität der getesteten Pilzstämme gegen *E. coli, B. velezensis* und *S. cerevisiae* wurde, nach einer Inkubationszeit von einem Tag bei 28°C, visuell überprüft. Eine gebildete Inhibierungszone um den platzierten Pilzstempel, zeigte das vorhanden sein von ASM gegenüber dem getesteten Mikroorganismus an. Pilzstämme, die gegen *F. oxysporum* getestet wurden, mussten bei 28°C, drei Tage lang inkubiert werden.

Die Ergebnisse zeigen, dass die Medienzusammensetzung einen Einfluss auf die ASM Produktion von Pilzen hat. Weiters spielt die Kultivierungszeit eine wichtige Rolle in der ASM Bildung. Verschiedene Pilzstämme zeigten eine Adaption von inhibierenden Stoffen, wenn diese als Co-Kulturen verwendet wurden. Die geringste inhibierende Konzentration konnte mit einigen SM Extrakten gegenüber *E. coli, B. velezensis* und *S. cerevisiae* getestet werden, jedoch zeigte kein SM Extrakt eine inhibierende Wirkung gegenüber *F. oxysporum*.

Penicillium expansum (TUCIM 5626), *Ovicillium* sp. (TUCIM 5628), *Xylaria* sp. (TUCIM 5712) und *Arthrinium rasikravindrii* (TUCIM 5773) können als beste ASM produzierende Stämme genannt werden und sollten mit NGS und -omics Technologie weiter erforscht werden.

Acronyms

ABR – Antibacterial resistance

AMR – Antimicrobial resistance

ASM – Antimicrobial secondary metabolites

Bv/B – *Bacillus velezensis*

COCY – Colobopsis cylindrica complex

Ec/E – *Escherichia coli*

Fo/F – *Fusarium oxysporum*

Malt Ex – Malt extract

MH – Müller-Hinton

MHA – Müller-Hinton Agar

MIC – Minimum Inhibitory Concentration

NGS – Next generation sequencing

PDA – Potato Dextrose Agar

Sc/S – *Saccharomyces cerevisiae*

SD – Standard deviation

SM – Secondary metabolites

WHO – World Health Organisation

YG – Yellow goo

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1 Introduction

Drug resistance is a serious problem and increasing globally. It is estimated to cause death of 700,000 to several million people per year. Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a result.[1] There are public calls for global collective action to address the threat that include proposals for international treaties on antimicrobial resistance. Worldwide antibiotic resistance is not completely identified, but poorer countries with weaker healthcare systems are more affected (Okeke *et al.*, 1999).

A World Health Organization (WHO) report released April 2014 stated, "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. Antibiotic resistance—when bacteria change so antibiotics no longer work in people who need them to treat infections—is now a major threat to public health."

The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928. Since then, antibiotics have transformed modern medicine and saved millions of lives. Antibiotics were first prescribed to treat serious infections in the 1940s (Deshmukh *et al.*, 2015). Penicillin was first tested on an Oxford policeman, who died after five days of treatment, due to shortage of Penicillin. More clinical studies had to be done to treat the civil people and soldiers in the World War II (Ligon 2004). However, shortly thereafter, penicillin resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened.

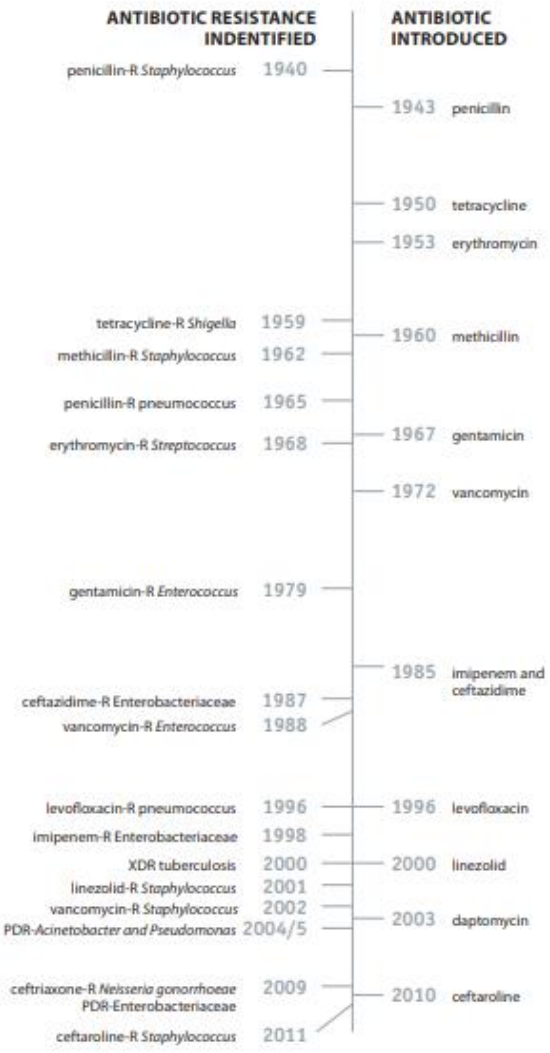


Figure 1: Developing Antibiotic resistance: A Timeline of Key Events [1]

[1] https://www.cdc.gov/drugresistance/index.html

In response, new beta-lactam antibiotics were discovered, developed, and deployed, restoring confidence (see Figure 1, Bush *et al.,* 1995). However, the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was identified during that same decade, in the United Kingdom in 1961 (Ayliffe 1997) and in the United States in 1968 (Enright *et al.,* 2002).

Unfortunately, resistance has eventually been seen to nearly all antibiotics that have been developed (Mathur and Singh 2005, De Francesco *et al.,* 2010, Baquero *et al.,* 1991). Vancomycin was introduced into clinical practice in 1972 for the treatment of methicillin resistance in both *S. aureus* and coagulase-negative staphylococci (Noble *et al,* 1992). It had been so difficult to induce vancomycin resistance that it was believed unlikely to occur in a clinical setting. However, cases of vancomycin resistance were reported in coagulase-negative staphylococci in 1979 and 1983 (Deshmukh *et al.,* 2015).

From the late 1960s through the early 1980s, the pharmaceutical industry introduced many new antibiotics to solve the resistance problem, but after that the antibiotic pipeline began to dry up and fewer new drugs were introduced. As a result, in 2015, many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat.

Among gram-positive pathogens, a global pandemic of resistant *S. aureus* and Enterococcus species currently poses the biggest threat (Stefani *et al.,* 2012). MRSA kills more Americans each year than HIV/AIDS, Parkinson's disease, emphysema, and homicide combined. Vancomycin-resistant enterococci (VRE) and a growing number of additional pathogens are developing resistance to many common antibiotics. (WHO Report 2014). The global spread of drug resistance among common respiratory pathogens, including *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, is epidemic.

Gram-negative pathogens are particularly worrisome, because they are becoming resistant to nearly all the antibiotic drug options available, creating situations reminiscent of the pre-antibiotic era. The emergence of multidrug resistance (and increasingly pan-resistant) gram-negative *bacilli* spp. has affected practice in every field of medicine (WHO Report 2014). The most serious gram-negative infections occur in health care settings and are most commonly caused by species belonging to family Enterobacteriaceae (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa,* and *Acinetobacter* (Nordmann *et al.,* 2009). MDR gram-negative pathogens are also becoming increasingly prevalent in the community. These include extended-spectrum beta-lactamase-producing *Escherichia coli* and *Neisseria gonorrhoeae*. Infections by fungi are a cause of high morbidity and mortality in immunocompromised persons, such as those with HIV/AIDS, tuberculosis or receiving chemotherapy (Verhoeff 1974, Kauffmann and Hedderwick 1997, Siqueira and Sen 2004).

Candida spp., *Cryptococcus neoformans* and *Aspergillus fumigatus* are reason behind most of the infections caused by fungi and antifungal resistance occurs in all of them (Karkowska-Kuleta *et al.,* 2009, Hsueh *et al.,* 2005). Multidrug resistance in fungi is increasing because of the widespread use of antifungal drugs to treat infections in immunocompromised individuals. Of particular note, Fluconazole-resistant *Candida* spp. have been highlighted as a growing problem by the CDC.

More than 20 species of *Candida* can cause Candidiasis infection, the most common of which is *Candida albicans*. *Candida* yeasts normally inhabit the skin and mucous membranes without causing infection. However, overgrowth of *Candida* can lead to Candidiasis. Some *Candida* strains are becoming resistant to first-line and second-line antifungal agents such as azoles and echinocandins (Sanglard *et al.,* 1995).

The discovery of penicillin in 1928 and other antibiotics in the 20th century proved to be a significant medical achievement, saving millions of lives and significantly reducing the burden of infectious diseases. However, the years between 1950s to 1970s represented the golden age of antibiotic discovery, where countless new classes of antibiotics were discovered to treat previously incurable diseases such as tuberculosis and syphilis (Schlaegel and O'Conner 1981, Turner *et al.,* 1969). However, since that time the discovery of new classes of antibiotics has been almost non-existent and represents a situation that is especially problematic considering the resiliency of bacteria shown over time and the continued misuse and overuse of antibiotics in treatment.

More and more bacteria are developing defence against antibiotics, thereby becoming resistant to treatment. This will lead to simple infections becoming lethal once again. Our need for new antibiotics is urgent.

It is well known that fungi remain one of the most important resources for the discovery of new bioactive compounds. It is thought that fungi rank as the second biggest kingdom of organisms in nature and that as many as 1.5–5.1 million fungal species exist. From the history of drug discovery from microorganisms, fungal secondary metabolites have provided a number of important drugs, such as the antibiotic penicillin, the immunosuppressant cyclosporine (Hiestand *et al.,* 1986) and the antihypercholesterolemic agents lovastatin and compacting (Kawaguchi *et al.,* 2013).

In their quest for new antibiotics, Nielsen *et al.,* (2017) sequenced the genomes of nine different types of Penicillium species and they found that the fungi have enormous, previously untapped potential for the production of new antibiotics and other bioactive compounds.

New antibiotics need to be developed to treat infections by pathogens that have evolved resistance to currently available antibiotics. Bioprospecting for natural products is a route for the discovery of sources of new drugs via the isolation of bioactive metabolites from living organisms.

Almost 90 years after the discovery of Penicillin, scientists all over the world continue to investigate natural products (Clardy et al., 2006, Hemaiswarya *et al.,* 2008). According to Newman and Cragg (2016), in the years 1981–2010, ~50% of all small molecules originated from natural products. Mainly antibacterial, antiviral and antifungal compounds have been developed from natural sources such as bacteria, virus and fungi themselves.

Diverse chemical compounds with equally diverse scaffolds and bioactivities have been reported from fungi over the years, the vast group still remains to be fully exploited as in today´s date out of ~1 million different fungal species only ~100,000 have been described.

Table 1: Common antibiotic producers and their activity

Antibiotic	***Producer organism***	***Activity***
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi
Gentamicin	*Micromonospora purpurea*	Broad spectrum

Phyllosphere fungi, which inhabit tissues of living plants, have been recognized to harbour tremendous species diversity and play important ecological roles (Blackwell 2011). Almost all major lineages of land plants distributed from polar regions to tropics are associated with taxonomically diverse phyllosphere fungi and can be subject to some ecological effects by these fungi, such as pathogenic damage or benefits of enhancing tolerances against herbivores or pathogens (Whipps *et al.,* 2008).

Accumulative studies on species diversity and community structure of phyllosphere fungi in forests– which present the ecosystem with the greatest biomass, productivity, and species diversity on Earth– can contribute to the detection of novel bio-resources.

Endophytes are microorganisms that live inside the living plant tissues for at least part of their life without causing any apparent disease symptoms in the host. It is estimated that each and every of the almost 300,000 plants that exist, hosts one or more endophyte. They occur everywhere, from the Arctic to Antarctic and temperate to the tropical climates (Saikkonen *et al.,* 1998, Martinez-Klimova *et al.,* 2017).

Endophytes are treated as endosymbionts. Both endophytic bacteria and endophytic fungi can co-exist in a single host plant. Endophytes enter inside plants primarily through the roots and the aerial portions of plants, such as leaves, flowers, stems and cotyledons.

They are localized at the point of entry and can spread in the whole host plant body. After entering the host, they reside within cells or the intercellular spaces or in the vascular (tissue) system (Rodrigues *et al.,* 2000).

Recent studies have shown, that those endophytic organisms are abundant and diverse producers of bioactive secondary metabolites. Those co-existent of fungi with leaves of tropical plants are an especially exciting and relatively untapped source of novel compounds.

Strobel and Daisy (2003) showed in their study, that the endophytic fungi *Cryptosporiopsis quercina* produce several unique antimycotic, like cryptocandin and cryptocin, which are based on peptides and a tetramic acid, to defend the host plant against other plant-pathogens. (Wilson 1995, Rodriguez *et al.,* 2008).

They have been recognized as useful sources of bioactive secondary metabolites (Higginbotham *et al.,* 2013). Metabolites isolated from the fungal endophytes i.e. alkaloids, terpenoids, quinines, isocoumarin derivatives, flavanoids, phenols, peptides and phenolic acids are good sources of novel antibiotics, immunosuppressant and anticancer compounds having diverse structural groups and showing antibacterial, antifungal, anticancer, antiviral, antioxidant, insecticide, antidiabetic and immunosuppressive activities (Clay 1988). The antibiotic resistance is a problem, which is accelerated by the mis- and overuse of antibiotics all over the world. One possible loophole could be to screen new antibiotics or antibiotic producers.

Figure 2: Island of Borneo, separated by three countries and covered mainly by rainforest [2]

Each year, thousands of new species are discovered by researchers around the world, mainly in the undiscovered parts of the rainforests and deep sea.

The island of Borneo, which is the third largest island on the world, with an area of 743.122 square kilometres, is separated by three different countries: 1) Malaysia (states of Sabah and Sarawak), 2) Brunei (Sultanate) and 3) Indonesia (Kalimantan – West, Central, South, and East), which cover 26,7%, 0,6% and 72,6% of the island mainland. This island is covered by 50% rainforest that contains a high biodiversity of 222 mammalian species (44 of which are endemic), 420 resident birds (37 endemic), 100 amphibians, 394 fish (19 endemic) and 15.000 plants (6.000 endemic).[2]

[2] https://data.mongabay.com/borneo.html

Several years ago, a new ant species was discovered by a team of seven research groups in the rainforest of Borneo (*Colobopsis explodens),* the name is related to their behaviour, when they were attacked by enemies (Zettel et al., 2018).

It is a suicide mission, where the ants sacrifice themselves to protect the rest of the colony, by attacking the enemy, angle their backsides in close and flex so hard that their abdomens burst at the seams. Those fights are mainly territory confrontation, to protect food resources and the colony against other ants or small insect (Laciny *et al.,* 2018).

Figure 3: *Colobopsis explodens* (small ant) protecting their colony against an enemy (big ant) by exploding and revealing a yellow sticky substance (goo) [3]

The symbioses between fungus-farming ants (Formicidae: Attini, ~ 200 species) and their fungi, which are the primary food source for the ants, can be dated back at 50 million years ago. (Mueller *et al.,* 1998). Although attini ants evolved all from one ancestor, extant species cultivate multiple, phylogenetically distant lineages of fungi, which are cultivated in monocultures and manured by the ants with plant substrates, insect frass or seeds (Currie *et al.,* 1999). Foundress queens of these species establish new colonies by digging chambers in the soil, expelling the fungal pellet that they bring from their natal nest, and initiating the cultivation of their own gardens, which are started by using faecal material provided by the queen (Lucas *et al.,* 2017). In this relationship, the attini ants control cultivar growth of the fungi garden and remove weedy fungal competitors to maintain healthy farms. In return, the fungi produce lipid and carbohydrate rich hyphae, known as gonglyidia, which are harvested by the ants and used as food (Seipke *et al.,* 2011). To prevent the garden against mites and nematodes, common invaders, farming insects are used, to down regulate the feeding of the invaders and to prohibit a contamination with “alien” spores. All this effort of stable growth condition occasionally attracts specialized fungal garden parasites, like the genus *Escovopsis* (Reynolds and Currie 2004). This fungal parasite slows down the growth of the fungal farm and reduce the crop productivity. To combat *Escovopsis* infections, the attini ants use natural antimicrobial substances, which are produced by some sort of bacteria (mainly genus *Streptomyces, Amycolatopsis*), that grow on leaves and are specific selected by the ants (Currie *et al.,* 1999) The compounds, which are produced by attine ant associated bacteria are mainly unknown, except of two substances: dentigeriumycin and candicidin.

This bacteria genus is already well-known and used in pharmacology industries for discovery of the antibiotics. (Mueller and Gerardo 2002; Barke *et al* 2010). To investigation the diversity and possible application of the fungi related to ants, some samples were taken from leaves, soil, and the ant nests of the ant's territory and transferred to TU Wien. [3]

[3] https://animals.howstuffworks.com/insects/exploding-ants-kill-with-toxic-goo.htm

2 Aim and tasks of the study

The aim of the thesis was setting up a pipeline to explore the antimicrobial activity of novel and rare fungi, isolated from the high canopy of Borneo rainforest.

To achieve this aim, several tasks were designed:

- To explore fungi from an unstudied habitat
- To screen different nutritional conditions for their potential to induce secondary metabolite (SM) production
- To test the ability of fungi to produce SM in stress conditions:
  - Co-culture (confrontations)
  - Starvation
- To prepare a library of SM extracts after lyophilization
- To characterise synergy of SM extracts
- To test the Minimum Inhibitory Concentration (MIC) of antimicrobial compounds in 96 deep well microplates

3 Materials & Methods

3.1 Tested strains

To investigate the diversity and possible application of the fungi related to ants, some samples were taken from leaves, soil, and the ant´s nests and transferred to TU Wien.
40 fungi strains were selected from out of 500 strains samples from the habitat of the exploding ants in the rainforest of Borneo and stored at TUCIM collection (see Table 2). The ITS region was chosen to identify fungal species, as it is well accepted phylogenetic barcode marker for fungi (Schoch *et al.,* 2012). We selected those strains, because they aren´t very common and less information is known, or are known as producer of bioactive metabolites, such as *Penicillium*, *Arthrinium* and *Trichoderma* (Hoff *et al.,* 2009, Ghisalberti *et al.,* 1991).

Table 2: Selected sample pool for testing the antimicrobial activity from the high canopy of Borneo rain forest

TUCIM	Origin	Putative identification	ITS coverage/Identity [%]	TUCIM	Origin	Putative identification	ITS coverage/ Identity [%]
5490	YG foraging, COCY	*Trichothecium* sp.	100/92	**5717**	Leaf-phyllo	*Arthrinium* sp.	94/98
5491	YG foraging, COCY	cf. *Paracremonium* sp.	99/87	**5718**	Leaf-phyllo	*P. cairnsense*	99/99
5492	YG foraging, COCY	cf. *Stromatonectria* sp.	99/86	**5767**	Leaf-phyllo	*P. rudallense*	99/100
5494	YG foraging, COCY	*Penicilium* sp.	99/99	**5773**	Leaf-phyllo	*Arthrinium rasikravindrii*	97/100
5495	YG foraging, COCY	*Pestalotiopsis* sp.	93/99	**5779**	Leaf-phyllo	*Arthrinium* sp.	99/99
5501	Brunei - Beetle	*T. strigosellum*	98/99	**5786**	Leaf-phyllo	cf. *Setophoma* sp.	88/94
5502	Brunei - Beetle	*T. sparsum*	92/98	**5789**	Leaf-phyllo	*Phlebia acerina*	99/99
5594	Leaf-phyllo	*T. harzianum*	100/97	**5801**	Leaf-phyllo	cf. *Infundichalara* sp.	95/89
5626	Leaf-phyllo	*P. expansum*	99/99	**5803**	Leaf-phyllo	*Coniochaeta* sp.	99/99
5628	Leaf-phyllo	*Ovicillium* sp.	84/99	**5806**	Leaf-phyllo	cf. *Setophoma* sp.	88/94
5629	Leaf-phyllo	*Verticillium* sp.	98/99	**5807**	Leaf-phyllo	*Arthrinium* sp.	98/99
5633	Leaf-phyllo	cf. *Chaunopycnis* sp.	98/96	**5808**	Leaf-phyllo	*Setophoma* sp.	98/99
5634	Leaf-phyllo	*Fusarium* sp.	99/99	**5828**	Leaf-phyllo	cf. *Leotiomycetes* sp.	85/88
5635	Leaf-phyllo	cf. *Valsaceae* sp.	94/91	**5830**	Leaf-phyllo	*Penicilium* sp.	97/100
5643	Leaf-phyllo	*Trichoderma* sp.	100/99	**5831**	Leaf-phyllo	*P. caseifulvum*	100/99
5646	Leaf-phyllo	cf. *Nemania* sp.	99/95	**6037**	Leaf-phyllo	*Lasiodiplodia* sp.	99/99
5650	Leaf-phyllo	*P. glabrum*	100/99	**6183**	n/a	cf. *Capnodium* sp.	99/95
5711	Leaf-phyllo	*Nemania* sp.	99/99	**6986**	n/a	cf. *Schwanniomyces vanrijiae*	96/96
5712	Leaf-phyllo	*Xylaria* sp.	97/99	**6987**	n/a	-	-
5716	Leaf-phyllo	*Leotiomycete* sp.	99/99	**6992**	n/a	-	-

Fungi from putatively new taxa are marked by brown colour; cf. means con forma indicating uncertain molecular identification due to the low similarity to known taxa.

3.2 Antimicrobial activity tests

3.2.1 Evaluation of the different growth conditions on antimicrobial metabolite production

The aim of this part was to find the best conditions, such as media, incubation time and stress conditions on antimicrobial metabolite production.

3.2.1.1 Finding optimal media

To find an ideal culture medium that could be applied to an antibiotic discovery, ten solid state media were chosen. After that, strains from Table 2 were cultivated by transferring the mycelia from the main strain with an inoculation loop or a needle to medium plates.

Table 3: Preparation of media with different nutritional composition

Media	*Media composition (1 L)*
1) Potato Dextrose Agar (PDA)	*4 g Potato extract, 20 g Dextrose, 15 g Agar*
2) YM Agar (YM)	*10 g Malt extract, 2 g Yeast extract, 15 g Agar*
3) Czapek Dox Agar (Dox)	*5 g Yeast extract, 30 g Sucrose, 2 g Sodium nitrate, 1 g Dipotassium phosphate, 0,5 g Magnesium sulphate, 0,5 g Potassium chloride, 0,01 Ferrous sulphate, 15 g Agar*
4) Malt Extract Agar (Malt Ex)	*20 g Malt extract, 1 g Peptone, 15 g Agar*
5) *YESD*	*20 g Soy peptone, 20 g Dextrose, 5 g yeast extract, 15 g Agar*
6) YPSS	*4 g Yeast extract, 14 g Soluble starch,* *1 g Dibasic dipotassium phosphate,* *0,5 g Magnesium sulphate heptahydrate, 5 g Agar*
7) PYG	*1,25 g Soy peptone, 1,25 g Yeast extract, 5 g Glucose, 15 g Agar*
8) *YES*	*20 g Yeast extract, 20g Sucrose,* *0,5 g Magnesium sulphate heptahydrate, 15 g Agar*
9) Oat Flour Agar	*30 g Oat flour, 15 g Agar*
10) Rice	*5 g Natural bio rice, 10 g corn meal, 10 g Agar*

The plates were incubated for one week at 28°C, darkness. During the incubation, the fungi started to grow and secrete metabolites into the cultivated medium.

Four model organisms (see Table 4) including gram-negative, gram-positive bacteria, a yeast and a filamentous fungus were chosen, for testing the potential antimicrobial activity.

Table 4: TUCIM of used model organisms

Model organism name	*TUCIM*
Escherichia coli	7221
Bacillus velezensis (Bv)	5484
Saccharomyces cerevisiae (Sc)	4081
Fusarium oxysporum (Fo)	4812

Of those four model organisms, cell suspensions were prepared, by adjusting the OD to 0,1 (at 600 nm) and inoculated with a cotton swab on Müller-Hinton Agar plates (MHA). [4]

Balouri *et al.*, (2016) described a way to check the antimicrobial metabolite ability of the tested organism in a qualitatively way. With a plug cutter, four small plugs (Ø =4 - 7 mm) were sliced in the plates from 3.2.1.1 and transferred to the inoculated Müller-Hinton plates with a needle by putting the agar side of the strain plug to the surface of inoculated MHA and the plates were incubated at 28°C.

The outcomes were recorded based on presence or absence of a clear inhibitory zone around the plugs, called “halo”. (see Figure 4).

Figure 4: Appearing halo indicates the growth inhibition of *B. velezensis* (left, 5712 (Dox), with 4 mm Ø plug), *E. coli* (middle, 5712 (Dox), with 4 mm Ø plug) *and S. cerevisiae* (right, 5492 (Rice-extract), with 5,5 mm Ø blank disk) on MHA

[4] Recipe: Müller-Hinton Agar (MHA) 2g Beef extract, 17,5 g Casein peptone, 1,5 g starch, pH 7,5, 18 g Agar for 1 L

3.2.2 Testing the antimicrobial activity of fungal secondary metabolites

3.2.2.1 Extraction of the secondary metabolites

To extract the secondary metabolites from fungi, the 40 strains were cultivated on rice in 250 mL Erlenmeyer flasks and in big petri dishes with three selected medium from previous test (PDA, Dox, Malt ex) and incubated at 28°C with a 12 h light/dark cycle.

For the rice media, approximately 10 g natural bio rice were weighed, filled up with 15 mL water and put inside the autoclave. The cultivation of diverse fungi on rice medium is a standard method to induce the antimicrobial metabolite production (Robinson et al., 2001, VanderMolen *et al.,* 2013).

The incubation time were depending on the growth rate of the fungi. After the strains cover all the flasks and plates, by use of spatula, they were cut into cubes and transferred into standing falcons.

For the following lyophilization, the samples had to be frozen, therefore, they were placed into the freezer (-80°C) for 1 hour. During that time, the lyophilization machine (Labconco FreeZone 2.5) was turned on and waited till it reached -47°C. The next step was to close the frozen samples with parafilm, where holes with a needle were created. Finally, the samples were placed into the lyophilization machine for two days and the lyophilization was started by using the vacuum pump (pressure ~ 0,100 mbar).

After two days, the lyophilization was stopped by opening one valve of the lid to reach normal pressure again. Then each freeze - dried sample was filled with 40 mL of a methanol/dichloromethane (1:2) solution and shaken for two hours. Properties of an efficient solvent in extraction include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate (Ncube *et al.*, 2008). The used mixture included methanol, which should extract the polar - and dichloromethane, which extracts the non-polar compounds. The extracts were separated from the media into new falcons afterwards by using Whatman filter papers, Ø = 70 mm.

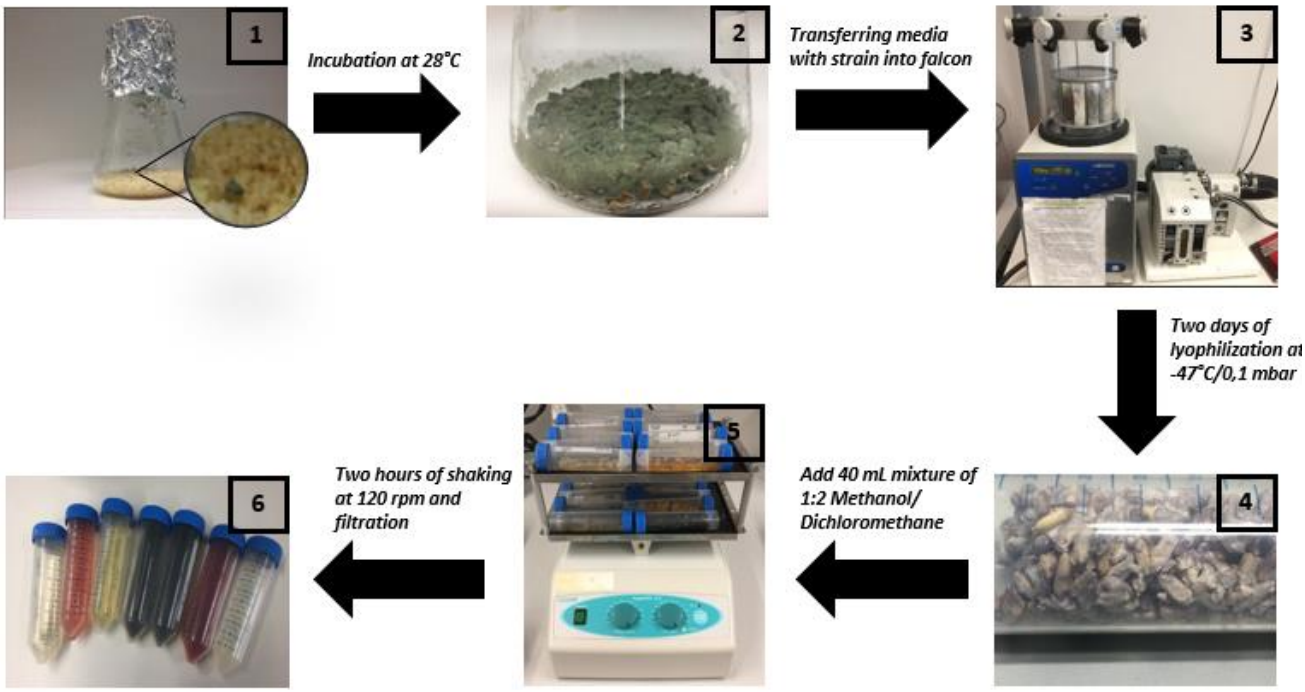


Figure 5: 1) Inoculation of sterile rice with strain plugs and incubated at 28°C without light, 2) overgrowing of strain on rice media, ready to use for lyophilization, 3) lyophilization at -47°C/0,1 mbar for two days, 5) dried media with strain were mixed with 40 mL of 1:2 methanol/dichloromethane solution and 5) put on a shaking board for two hours at max speed 6) extracts were separated from media and other solid substances by pouring extracts over a filter paper into 50 mL standing falcons

For synergy and minimum inhibitory concentration experiments, 5 mL of the prepared extracts were transferred into a glass plate and kept in the chemical hood until all the solvent were evaporated. Then, to dissolve the dried extract, 3 x 500 µL methanol were rinsed over the glass plate and collected in 1,5 mL microtube and keep the tubes open in the hood till the methanol evaporated. The extracts were then weighed in the tubes and diluted with methanol to a final concentration of approximately 20 mg/mL.

3.2.2.2 Determination of the antimicrobial activity of the fungal secondary metabolites

The base of the test was similar to previous experiment with agar plugs on MHA (see 3.2.1.1). First MHA were inoculated with the four model strains. To prepare the extract disks, 10 µL extracts were pipetted placed on sterile paper disks (were made by punching of whatmann filter paper No 1 and autoclaved) and waited for 5 min until the methanol evaporated. The disks were placed on the inoculated MHA plates and incubated for one day for bacteria and two days for two model fungi strains. The results were recorded base on production of inhibition halo around the disks (Balouri *et al.,* 2016).

3.2.3 Determination of Minimum Inhibitory Concentration (MIC) of the fungal secondary metabolites

Broth microdilution is one of the most basic antimicrobial susceptibility testing methods. Briefly, the procedure involves preparing two-fold dilutions of the antimicrobial agent (e.g. 1, 2, 4, 8, 16 and 32 µg/mL) in a liquid growth medium dispensed in 96-well microtitration plate. Then, each well is inoculated with a microbial inoculum and inoculated under suitable conditions. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in microdilution wells as detected by the unaided eye. Broth dilution has been standardized by clinical and laboratory standards institute (CLSI) and we followed that standard protocol (Eloff 1998).

Broth Microdilution Procedure

1. A single 96-well microdilution plate can be used for up to four compounds. Each drug should be tested in duplicate. (Drug 1: rows A, B; Drug 2: rows C, D; Drug 3: rows E, F; and Drug 4: rows G, H). Wells 1 was used as blank and sterility control. Wells 2 - 11 will be sufficient to test the number of dilutions necessary for an end point MIC. In our experiment we were used 10 dilution of the extracts to test against the four model organisms (1024 - 2 µg/mL). Wells 12 for growth controls.
2. 200 µL of MHB were pipetted to wells 1 of the microdilution plate (as blank and sterility control)
3. 100 µL MHB were added to wells 3-12
4. 179.5 µL of MHB and 20.5 µL extract stock of the extracts (concentration 20 mg/mL in methanol) were pipetted to wells 2.
5. Dilute the extract in serial two-fold dilutions using a 100 µL multichannel pipette, beginning at the second well and continuing through well 11. Discard the final 100 µL of extract solution. Well 12 were served as the growth control.

Preparation of the tested microbial models

1. The cell suspensions were prepared, like in point 3.2.1.1. Young colonies of the bacteria and *S. cerevisiae* strains were added to sterile glass tubes, filled with 10 mL Müller-Hinton Broth (MHB) to adjust the OD 0,1 at wavelength of 600 nm by using turbidimeter (Biolog Model number 21907). For *F. oxysporum*, a dense spore suspension was prepared, filtered through an Eppendorf tube with sterile glass wools and used to make the working suspension. This OD is equal to 1 - 1.5 × 108 Colony forming unite (CFU)/mL bacteria and 1 – 5 × 106 CFU *S. cerevisiae* and *F. oxysporum* spores. For the bacteria and fungi, the cell suspensions were diluted in MHB 1000 and 100 times respectively (to obtained approximately 105 CFU/mL bacteria and 104 CFU/mL fungi).
2. 100 µL of the cell suspensions were transferred to the wells 2 - 12.

3. The final volume of all wells will be 200 µL after that step and the concentration of the extracts and bacteria numbers were reduced 2 - fold (we were added 100 µL of the cell suspensions to 100 µL of extract dilutions). The final concentration of the extracts were 1024, 512, 258, 124, 64, 32, 16, 8, 4, 2 µg/mL.
4. The plates were incubated at 28°C.
5. The MIC of the plates were recorded after 18, 24 and 48 hours of incubation visually and by spectrophotometer at 600 nm.

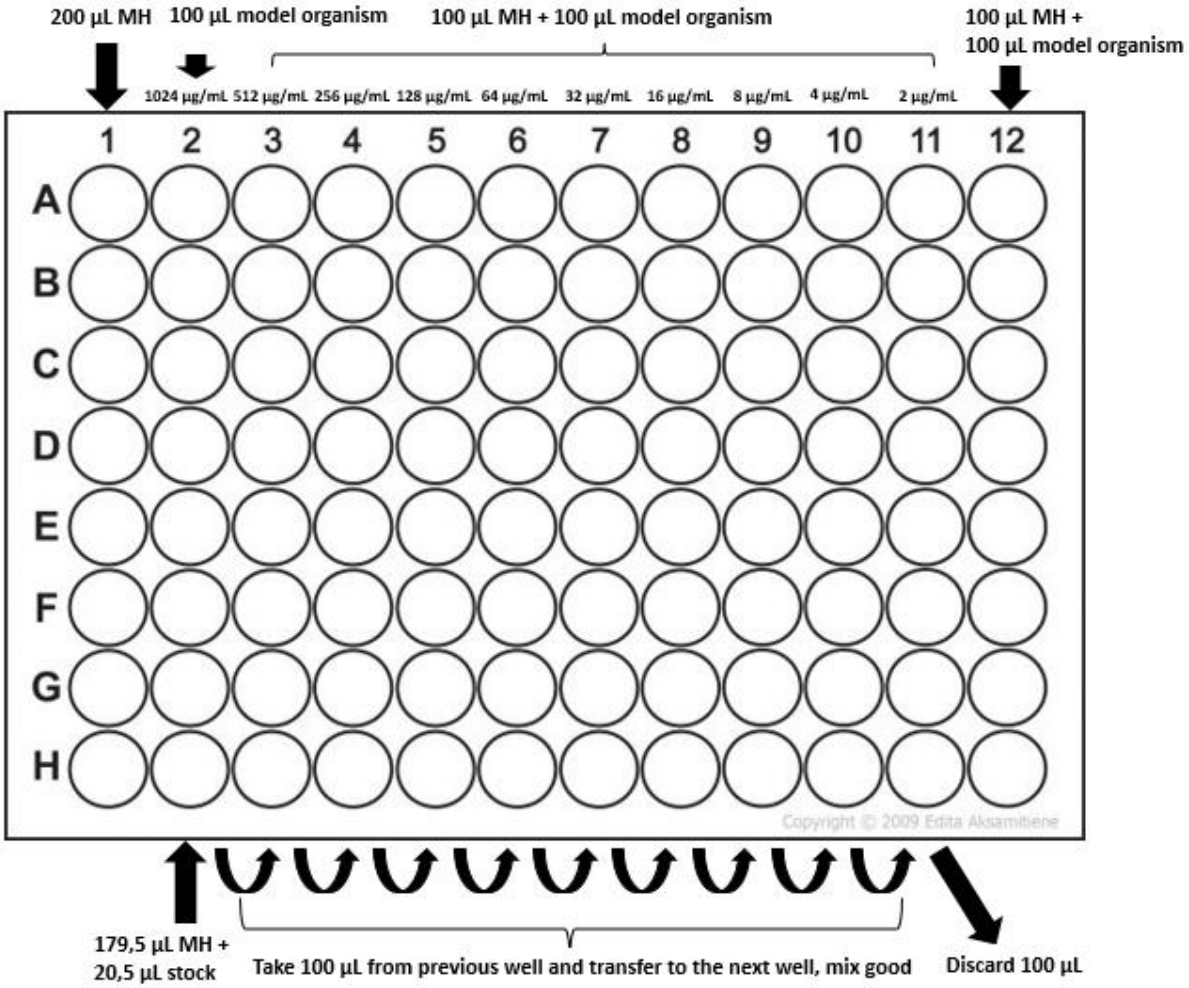


Figure 6: Sketch of MIC experiment on 96 deep well microplates

3.3 Inducing secondary metabolite production under stress condition

3.3.1 Dual confrontation assay

The idea behind this experiment was that the production of some secondary metabolites is not constitutive, and they may induce by some other factors, like stress condition or in competition with other organisms (Oh *et al.,* 2007). Therefore, we were selected all the positive and suspicious strains from test 3.2.1.1 and confronted against each other (see Figure 7). The nutrition of those plates had the same composition as the medium from the plates where the samples were selected (incubated at 28°C). The bioactivity was tested as shown in point 3.2.1.1.

Figure 7: Dual confrontation of strain 6037 and 5712 on Malt Ex

3.3.2 Starvation

The idea for this experiment was, that the secondary metabolite production could be induced by starving the strains, as another stress factor. The strains were cultivated as shown in 3.2.1.1 and after certain time points, the bioactivity was tested as explained in 3.2.1.1, to see, if the production of secondary metabolites was induced or switched off (see Figure 8).

Figure 8: Observation of *Arthrinium rasikravindrii* growth on PDA over three weeks

3.3.3 Synergy of extracts

Sometimes the mixture of two metabolites increase the antimicrobial activities. For example, Trimethoprim/sulfamethoxazole and Quinuprisitin/dalfoprisitin are two commercial antibiotics and they consist of combination of two antibiotics and they are more effective than either of its components individually in treating bacterial infections. In this experiment, we were tested the antimicrobial activity of the mixture of the fungi secondary metabolites and compared them with their individual activities. Therefore, 5 µL of two secondary metabolites were pipetted on paper disks and placed on incubated MHA plates, like written in point 3.2.2.2 and incubated at 28°C for one day for both bacteria organisms and three days for yeast and fungi organisms. The inhibitory halo size of the mixtures and the strains individually were compared and any synergistic or reduction of the halo size were recorded.

4 Results and discussions

4.1 Antimicrobial activity tests

4.1.1 Effect of the different nutritional conditions

In the next few points the results of antimicrobial activity of the 40 tested strains grown on 10 different media against four model organisms were summarized. Each table contains at top the four model organisms and on the left side the cultivated strains. Pictures were labelled with crosses (= negative) and checks (= positive), if an inhibitory halo appeared and therefore, indicated the antimicrobial compound production. But appearing halos of strains can´t be compared with each other to define the best producer for antimicrobial compounds, because the size and solubility of the compounds are different. For example, smaller non-polar compounds can diffuse faster through the media net than larger non - polar metabolites. All plates were incubated for one day at 28°C (except for the filamentous fungus *F. oxysporum,* incubated for three days).

4.1.1.1 PDA

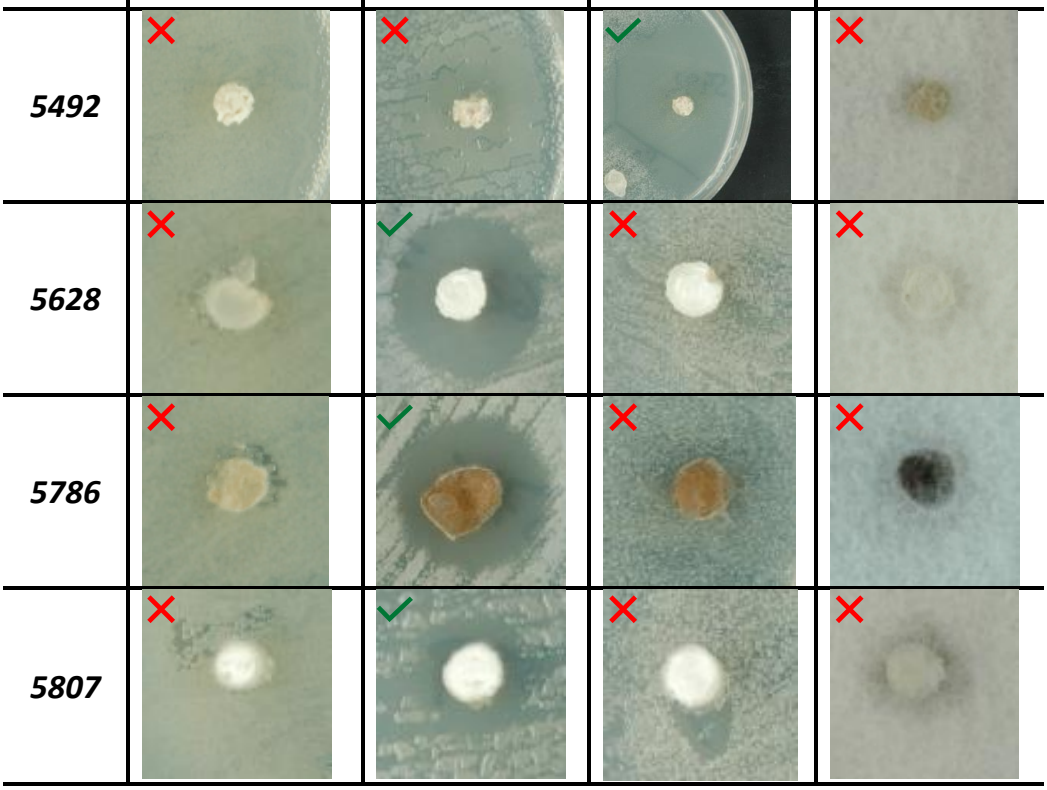
In Table 5 the results for the positive strains cultivated on PDA are presented. Out of 40 cultivated strains only four strains show an inhibitory activity against the model organisms.

Table 5: List of strains, cultivated on PDA and show antimicrobial activity against the four model organisms

TUCIM	*Identification*	*Ec*	*Bv*	*Sc*	*Fo*
5492	cf. *Stromatonectria* sp.	X	X	✓	X
5628	*Ovicillium* sp.	X	✓	X	X
5786	cf. *Setophoma* sp.	X	✓	X	X
5807	*Arthrinium* sp.	X	✓	X	X

Table 6: Summary of results for strains, grown on PDA and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc) and *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5492	✗	✗	✓	✗
5628	✗	✓	✗	✗
5786	✗	✓	✗	✗
5807	✗	✓	✗	✗



Note: 4 mm Ø plugs were used

4.1.1.2 YM

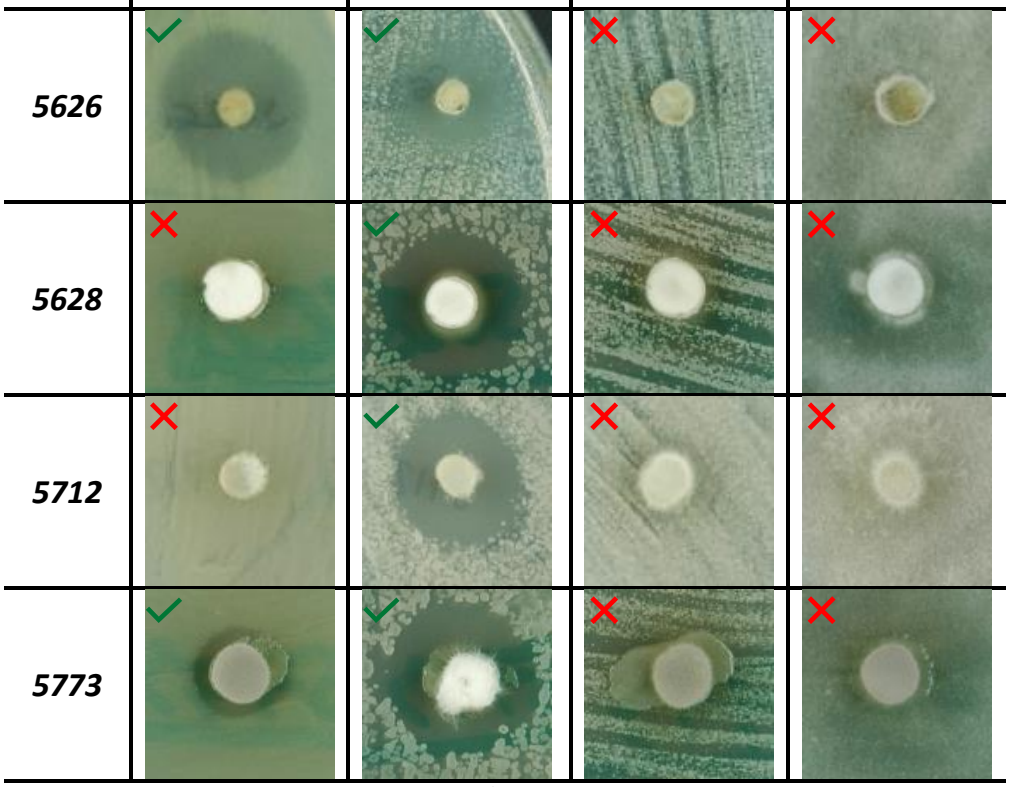
All four positive strains, out of a pool of 40 cultivated strains, which shows antimicrobial activity against the testing strains, are summarized in Table 7.

Table 7: List of strains, cultivated on YM and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5626	*P. expansum*	✓	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	X	✓	X	X
5773	*A. rasikravindrii*	✓	✓	X	X

Table 8: Summary of results for strains, grown on YM and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc) and *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5626	✓	✓	✗	✗
5628	✗	✓	✗	✗
5712	✗	✓	✗	✗
5773	✓	✓	✗	✗



Note: 4 mm Ø plugs were used

4.1.1.3 Dox

Out of 40 tested strains, eight strains show an inhibitory activity against the four testing strains, as shown in Table 9.

Table 9: List of strains, cultivated on Dox and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5491	cf. *Paracremonium* sp.	X	✓	X	X
5626	*P. expansum*	✓	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	✓	✓	X	X
5773	*A. rasikravindrii*	X	✓	X	X
5808	*Setophoma* sp.	X	✓	X	X
6986	cf. *Schwanniomyces vanrijiae*	X	✓	X	X
6992	-	✓	✓	X	X

Table 10: Summary of results for strains, grown on Dox and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc) and *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5491	✗	✓	✗	✗
5626	✓	✓	✗	✗
5628	✗	✓	✗	✗
5712	✓	✓	✗	✗
5773	✗	✓	✗	✗
5808	✗	✓	✗	✗
6986	✗	✓	✗	✗
6992	✓	✓	✗	✗

Note: 4 mm Ø plugs were used

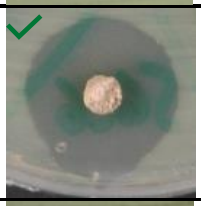
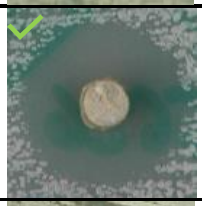
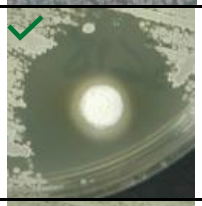
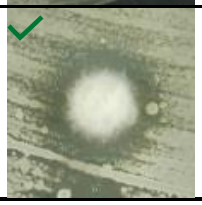
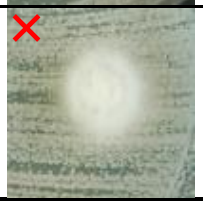
4.1.1.4 Malt Ex

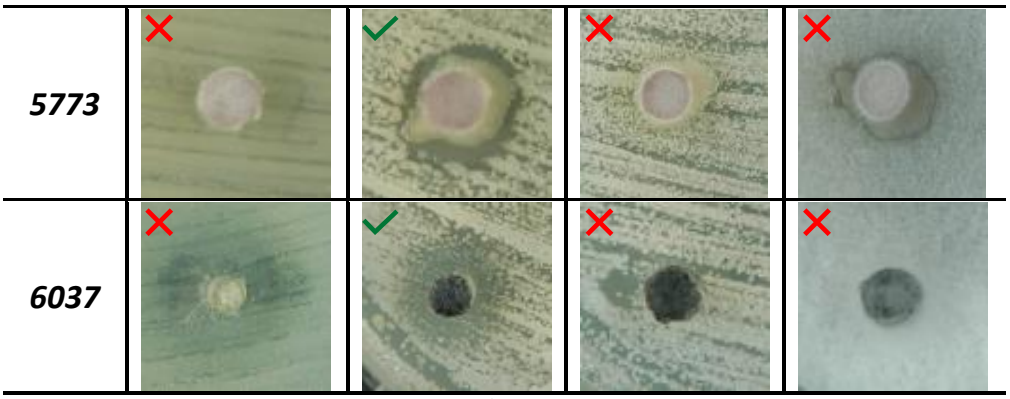
Results are summarized in Table 11 and were obtained out of a sample pool of 40 strains.

Table 11: List of strains, cultivated on Malt Ex and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5492	cf. *Stromatonectria* sp.	X	X	✓	X
5626	*P. expansum*	✓	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	X	✓	X	X
5773	*A. rasikravindrii*	X	✓	X	X
6037	*Lasiodiplodia sp.*	X	✓	X	X

Table 12: Summary of results for strains, grown on Malt Ex and tested against *E.coli (Ec)*, *B. velezensis (Bv)*, *S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5492				
5626				
5628				
5712				



Note: 4 mm Ø plugs were used

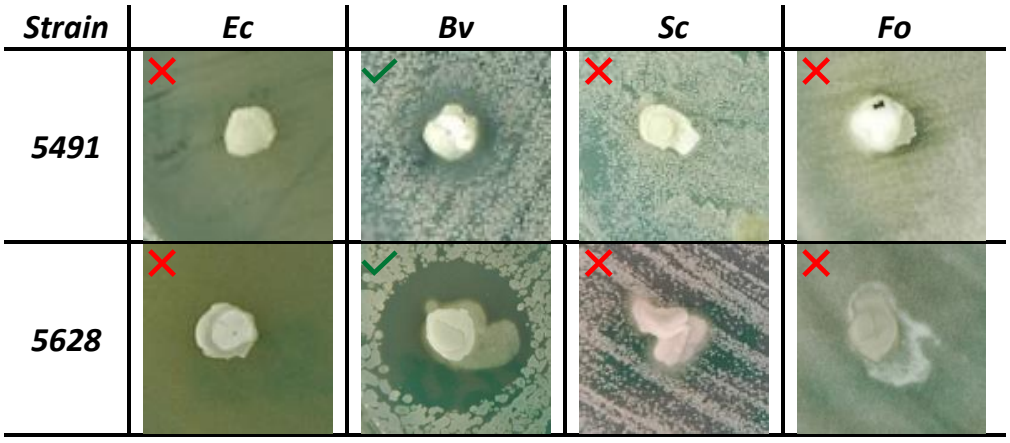
4.1.1.5 YPSS

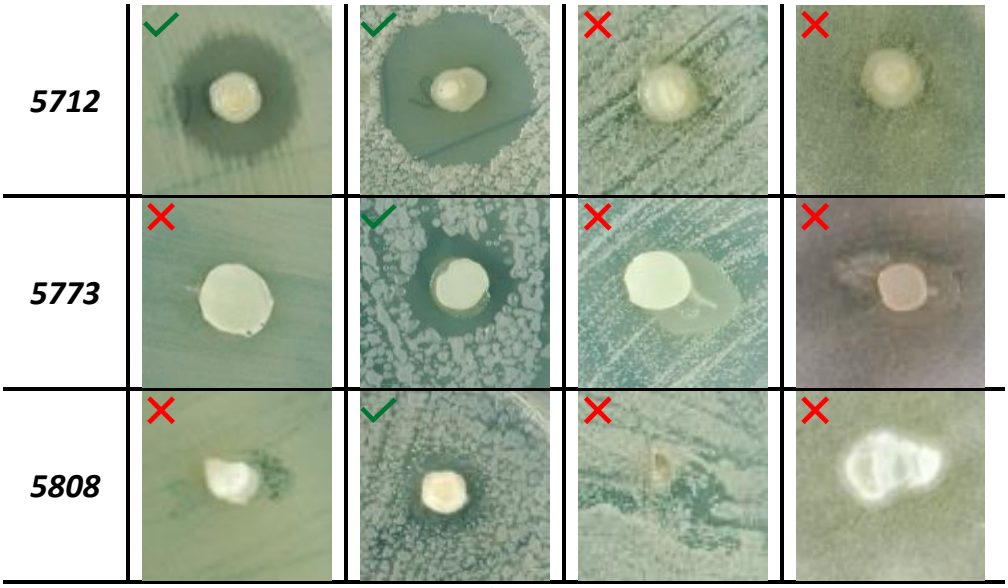
Table 13 shows five strains with antimicrobial activity against the four model organisms and tested out of 40 cultivated samples.

Table 13: List of strains, cultivated on YPSS and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5491	cf. *Paracremonium* sp.	X	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	✓	✓	X	X
5773	*A. rasikravindrii*	X	✓	X	X
5808	*Setophoma* sp.	X	✓	X	X

Table 14: Summary of results for strains, grown on YPSS and tested against *E.coli (Ec)*, *B. velezensis (Bv)*, *S. cerevisiae (Sc)* and *F. oxysporum (Fo)*





Note: 4 mm Ø plugs were used

4.1.1.6 YESD

Out of 40 cultivated samples, six strains (see Table 15) show an antimicrobial activity against the four testing strains.

Table 15: List of strains, cultivated on YESD and show antimicrobial activity against the four model organisms

TUCIM	*Identification*	*Ec*	*Bv*	*Sc*	*Fo*
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	✓	✓	X	X
5773	*A. rasikravindrii*	✓	✓	X	X
5808	*Setophoma* sp.	X	✓	X	X
5830	*Penicillium* sp.	X	✓	X	X
6986	cf. *Schwanniomyces vanrijiae*	X	✓	X	X

Table 16: Summary of results for strains, grown on YESD and tested against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5628	✗	✓	✗	✗
5712	✓	✓	✗	✗
5773	✓	✓	✗	✗
5808	✗	✓	✗	✗
5830	✗	✓	✗	✗
6986	✗	✓	✗	✗

Note: 4 mm Ø plugs were used

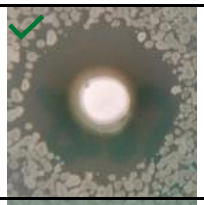
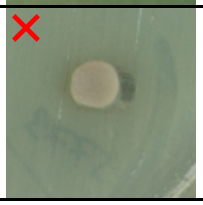
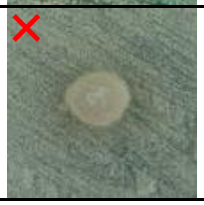
4.1.1.7 PYG

Table 17 shows the two antimicrobial compound producers out of 40 cultivated samples.

Table 17: List of strains, cultivated on PYG and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5628	*Ovicillium* sp.	X	✓	X	X
5773	*A. rasikravindrii*	X	✓	X	X

Table 18: Summary of results for strains, grown on PYG and tested against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5628				
5773				

Note: 4 mm Ø plugs were used

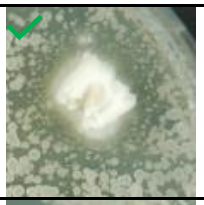
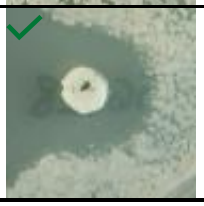
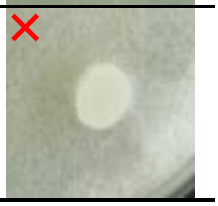
4.1.1.8 YES

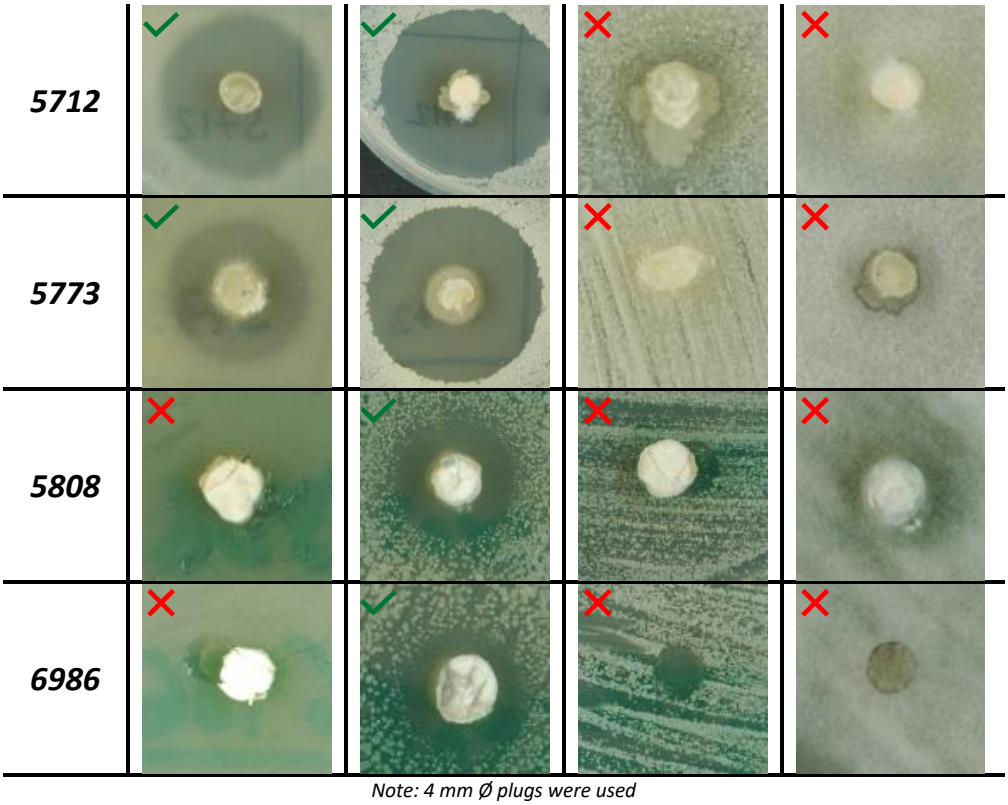
Six bioactive strains could be determined out of a pool of 40 samples, which can be seen in Table 19.

Table 19: List of strains, cultivated on YES and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5491	cf. *Paracremonium* sp.	X	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	✓	✓	X	X
5773	*A.rasikravindrii*	✓	✓	X	X
5808	*Setophoma* sp.	X	✓	X	X
6986	cf. *Schwanniomyces vanrijiae*	X	✓	X	X

Table 20: Summary of results for strains, grown on YES and tested against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5491				
5628				



Note: 4 mm Ø plugs were used

4.1.1.9 Oat Flour

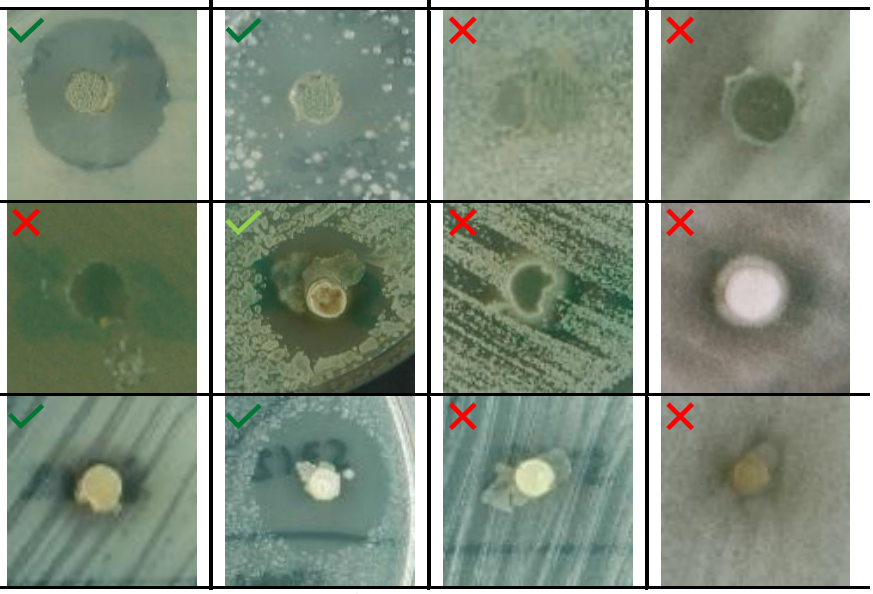
With this media, three positive (see Table 21) results for antimicrobial compound producer could be gained out of 40 cultivated samples.

Table 21: List of strains, cultivated on Oat Flour and show antimicrobial activity against the four model organisms

TUCIM	*Identification*	*Ec*	*Bv*	*Sc*	*Fo*
5626	*P. expansum*	✓	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5712	*Xylaria* sp.	✓	✓	X	X

Table 22: Summary of results for strains, grown on Oat Flour and tested against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5626	✓	✓	✗	✗
5628	✗	✓	✗	✗
5712	✓	✓	✗	✗



Note: 4 mm Ø plugs were used

4.1.1.10 Rice

For the last tested medium, six bioactive metabolite producers could be achieved out of a pool of 40 samples, as shown in Table 23.

Table 23: List of strains, cultivated on Rice and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5490	*Trichothecium* sp.	X	✓	X	X
5491	cf. *Paracremonium* sp.	X	✓	X	X
5626	*P. expansum*	✓	✓	X	X
5628	*Ovicillium* sp.	X	✓	X	X
5801	cf. *Infundichalara* sp.	✓	✓	X	X

Table 24: Summary of results for strains, grown on Rice and tested against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc)* and *F. oxysporum (Fo)*

Strain	Ec	Bv	Sc	Fo
5490	✗	✓	✗	✗
5491	✗	✓	✗	✗
5626	✓	✓	✗	✗
5628	✗	✓	✗	✗
5801	✓	✓	✗	✗

Note: 4 mm Ø plugs were used

4.1.1.11 Summary of different nutritional conditions

In Table 25 the result for all antimicrobial metabolite producers, cultivated on different nutritional conditions, are summarized. Base of the results, we cannot use only a few media to screen for production of antimicrobial compounds produced by fungi. In each medium the tested fungi strains were shown different antimicrobial properties. Also, base of the results, we can´t consider special nutrients from the tested medium compositions as an anti - microbial testing medium. From 40 tested strains, 15 of them have demonstrated inhibitory activities against at least one of the four model organisms in all ten media. Out of those ten media, we can select five, which can cover all of the antimicrobial activities of the fungi metabolites. They are included PDA and Malt extract (as best anti-fungal media) and Dox, YESD, Rice media.

Dox was the best medium in production of antibacterial agents against both tested bacteria strains and 50% (8 of 15 positive strains) of all the antimicrobial producers were shown inhibitory activity against them. The YPSS medium wasn´t used for the next experiments, because of its softness, which made it difficult to pick complete plugs for the experiments.

Table 25: Summary of all antimicrobial metabolite producers, cultivated on media with different nutritional conditions and tested against *E.coli* (E), *B. velezensis* (B), *S. cerevisiae* (S) and *F. oxysporum* (F)

		Different nutritional conditions																																	
		PDA				YM				DOX				MEA				YESD				YPSS				YES				PYG				OFA				RICE A			
		Potato, dextrose				Malt ex, yeast ex				Sucrose				Malt ex, Peptone				Soy peptone, Glucose				yeast ex, starch				Sucrose, Yeast ex				Soy peptone, yeast ex, Glucose				Oat flour				Rice, Corn meal			
TUCIM	**Identification**	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F	E	B	S	F
5490	*Trichothecium* sp.																																				x		
5491	cf. *Paracremonium* sp.										x												x				x										x		
5492	cf. *Stromatonectria* sp.			x												x																							
5626	*P. expansum*					x	x			x	x			x	x																			x	x			x	x		
5628	*Ovicillium* sp.		x				x				x				x				x				x				x				x				x				x		
5712	*Xylaria* sp.						x			x	x				x			x	x			x	x			x	x							x	x						
5773	*A.rasikravindrii*						x				x				x			x	x				x			x	x				x										
5786	cf. *Setophoma* sp.		x																																					
5801	cf. *Infundichalara* sp.																																					x	x		
5807	*Arthrinium* sp.		x																				x																		
5808	*Setophoma* sp.										x								x								x														
5830	*Penicillium* sp.																		x																						
6037	*Lasiodiplodia* sp.													x	x																										
6986	cf. *Schwanniomyces vanrijiae*										x								x								x														
6992	-									x	x																														
	Total	**0**	**4**	**2**	**0**	**1**	**4**	**0**	**0**	**3**	**8**	**0**	**0**	**2**	**5**	**1**	**0**	**2**	**6**	**0**	**0**	**1**	**5**	**0**	**0**	**2**	**6**	**0**	**0**	**0**	**2**	**0**	**0**	**2**	**3**	**0**	**0**	**2**	**5**	**0**	**0**

4.2 Determination of the antimicrobial activity of the SMs

The following tables Table 27 -Table 33 present the outcome of the freeze – dried samples, cultivated on four different media and extracted the antimicrobial compounds with a mixture of methanol/dichloromethane (1:2). Each table contains at top the four model organisms and on the left side the extracts of the freeze - dried strains. Pictures were labelled with crosses (= negative) and checks (= positive), if an inhibitory halo appeared and therefore, indicated the bioactive production. But appearing halos of strains can´t be compared with each other to define the best producer for bioactive compounds against the testing strain, because the identification of these compounds wasn´t done. All MHA testing plates were incubated for one day at 28°C (except for the testing strain *F. oxysporum,* incubated for three days).

The main idea for this experiment was, to determine, if antimicrobial compounds, which were produced in the different media, can be extracted and regained again.

4.2.1 PDA

As shown in Table 27, three antimicrobial metabolite producers could be tested with strain extract gained after cultivation on PDA with less agar. No bioactivity against *F. oxysporum* could be checked, but all strains, except for 5490, produce metabolites to inhibit the growth of *B. velezensis,* one also towards *S. cerevisiae*. To see, if the recovery rate for antimicrobial metabolites in extracts is the same as tested in point 4.1.1.1, both Table 6) and Table 27) were compared with each other.

The strains, that show inhibitory activity in Table 6 are: 5492, 5628, 5629, 5786 and 5807. After comparison with the results of Table 27, shows clearly, that only strain 5807 accorded with the five strains above. This could have multitude causes:

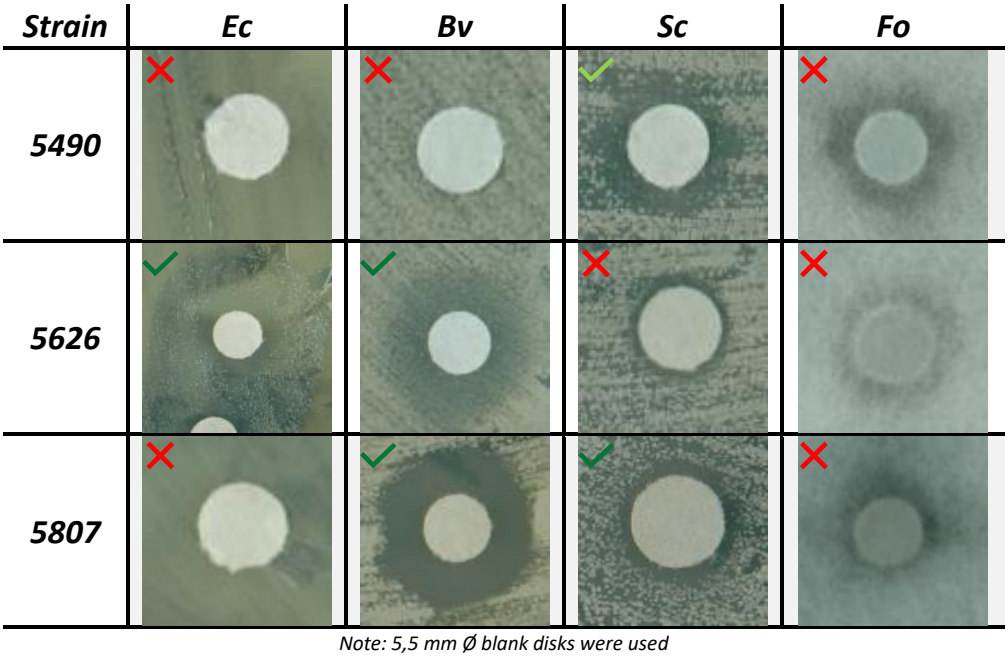
1) As shown in point 4.4.2.1, the chance, that antimicrobial metabolites against *B. velezensis* remain longer than two weeks of cultivation is higher, than for the rest of the antimicrobial metabolites.
2) The strains for Table 6 were always cultivated on media plates between one and two weeks and were than tested against the four testing strains. For Table 27, big plates were taken, and strains were cultivated till the plate were covered by the fungi. So, most of the time, the cultivation time was longer than for Table 6, which result in more positive strains, with inhibitory effect against *B. velezensis*.
3) The longer cultivation time, as explained above, could give the strains enough time, to produce antimicrobial metabolites, although the time wasn´t too long to degrade them again.

4) The extraction of the bioactive metabolites was done by using harsh conditions, which also can have an influence on this experiment. Also, only one solvent mixture was taken for the extraction and not several ones.
5) One big issue could also be the concentration of the active compounds, because when they were tested in point 4.1.1.1, the plugs were taken randomly out of the grown strain. So, the chance to have the same antimicrobial metabolite concentration in all four plugs is not as high, as when the extracts of the hole plate were tested.

Table 26: List of strain extracts, cultivated on PDA and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5490	*Trichothecium* sp.	X	X	✓	X
5626	*P. expansum*	✓	✓	X	X
5807	*Arthrinium sp.*.	X	✓	✓	X

Table 27: Summarized results for the extracts, gained after lyophilization of strains, cultivated on PDA and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)



Note: 5,5 mm Ø blank disks were used

4.2.2 Dox

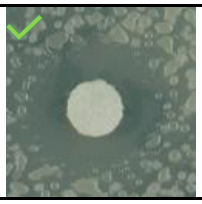
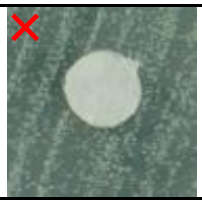
As shown in Table 29, only one antimicrobial metabolite producer could be tested, after lyophilization and extraction with methanol/dichloromethane (1:2). Compared with Table 9 where eight compound producer (5491, 5626, 5628, 5712, 5773, 5808, 6986, 6992) to inhibit the growth of the model organisms could be tested, here, only strain 5626, shows activity towards *E. coli* and *B. velezensis*.

The reasons, why only one strain could be tested after the lyophilization and extraction are manifold and can be seen in 4.2.1

Table 28: List of strain extracts, cultivated on Dox and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5626	*P. expansum*	X	✓	X	X

Table 29: Summarized results for the extracts, gained after lyophilization of strains, cultivated on Dox and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5626				

Note: 5,5 mm Ø blank disks were used

4.2.3 Malt Ex

In Table 31, the results for the extracts, gained after cultivation on Malt Ex and lyophilized and extracted with methanol/dichloromethane (1:2) are shown. As presented, two antimicrobial metabolite producers towards the four model organisms could be tested.

Compared to Table 11, where six strains (5492, 5626, 5628, 5712, 5773, 6037) show an inhibitory activity towards the testing strains, here, only two strains (5626, 6037) show an antimicrobial activity towards the testing strains. So, at least, half of the strains, which show inhibitory effect in Table 11, could be tested also, after lyophilization. Reasons, why not all strains could be checked towards their activity to inhibit the growth of the model organisms can be seen in 4.2.1.

Table 30: List of strain extracts, cultivated on Malt Ex and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5626	*P. expansum*	✓	✓	X	X
6037	*Lasiodiplodia sp.*	X	✓	X	X

Table 31: Summarized results for the extracts, gained after lyophilization of strains, cultivated on Malt ex and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5626	✓	✓	✗	✗
6037	✗	✓	✗	✗

Note: 5,5 mm Ø blank disks were used

4.2.4 Rice

In Table 33, the results for the extracts, gained after lyophilization of the strains, cultivated on rice medium and extracted with a mixture of methanol/dichloromethane (1:2), are summarized. Ten strains out of 40 strain samples, which show an inhibitory ability to stop the growth of *E. coli*/*B. velezensis* and *S. cerevisiae* could be tested. Unfortunately, no strain extract shows an inhibitory effect against *F. oxysporum*.

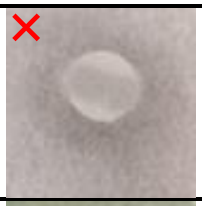
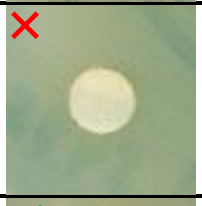
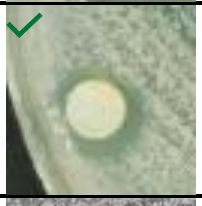
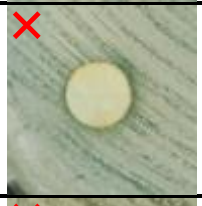
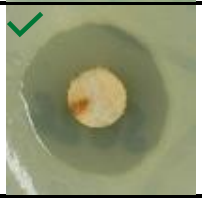
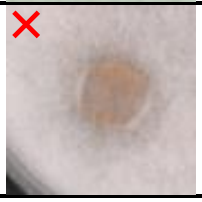
To see, if the recovery rate for bioactive metabolites in extracts is the same as tested in point 4.1.1.10, both Table 23) and Table 33) were compared with each other. The strains, which show an antimicrobial activity against the four model organisms in Table 23 are: 5490, 5491, 5626, 5628 and 5801.

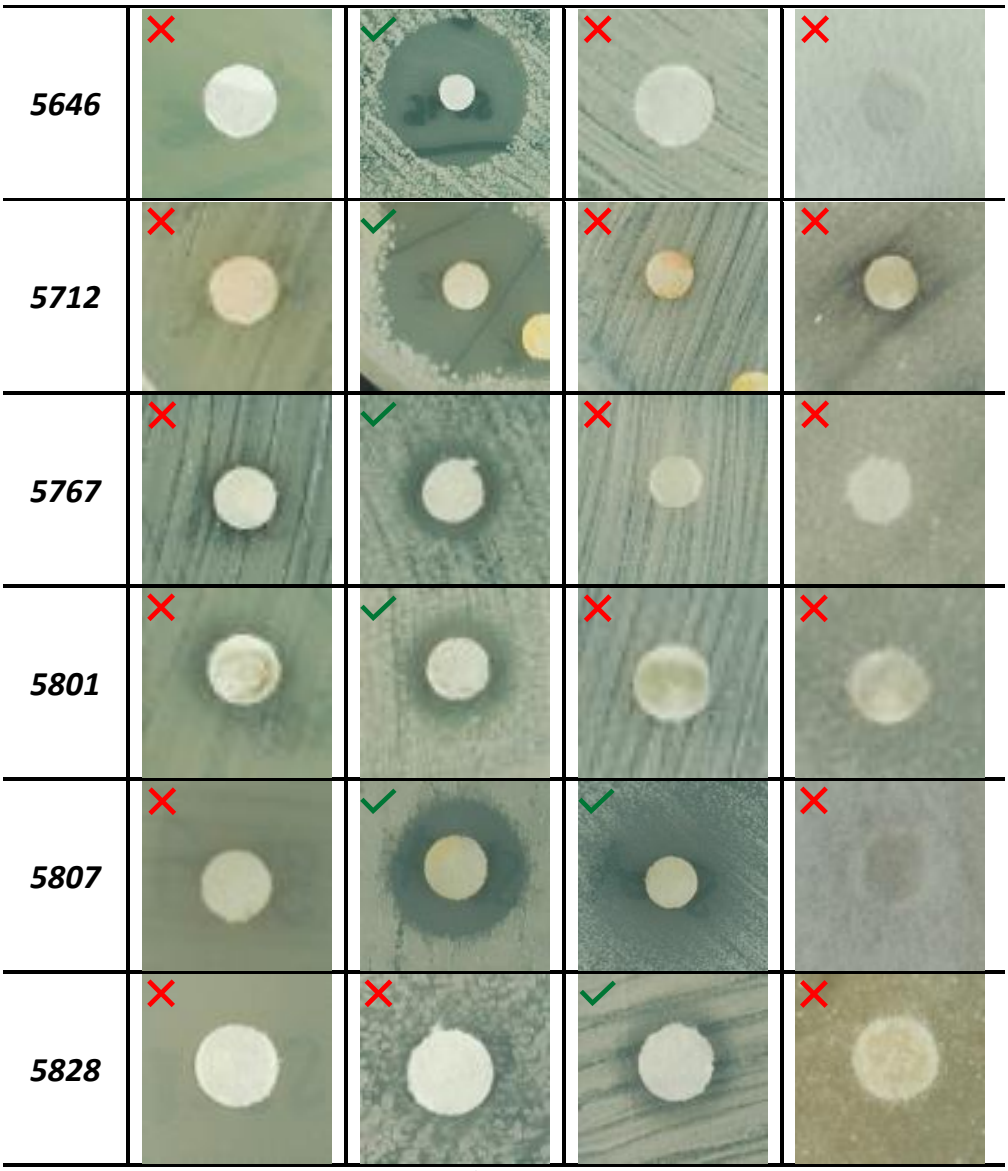
Compared to the results in Table 23, all strains, written above, except for strain 5628, also could be tested by using their extracts. Strain 5491 and 5626, show the same activity in both tables, but for strain 5490 an activity to inhibit the growth towards *Sc* could be achieved, by using the extracts, which is lost towards *E. coli* by strain 5801. So, by using the extracts, ten antimicrobial metabolite producers could be tested in total, compared to the five strains, which were tested in Table 23. This could have multitude causes, as you can see in 4.2.1.

Table 32: List of strain extracts, cultivated on Malt Ex and show antimicrobial activity against the four model organisms

TUCIM	Identification	Ec	Bv	Sc	Fo
5490	*Trichothecium sp.*	X	✓	✓	X
5491	cf. *Paracremonium sp.*	X	✓	X	X
5495	*Pestalotiopsis sp.*	X	✓	X	X
5626	*P. expansum*	✓	✓	X	X
5646	cf. *Nemania sp.*	X	✓	X	X
5712	*Xylaria sp.*	X	✓	X	X
5767	*P. rudallense*	X	✓	X	X
5801	cf. *Infundichalara sp.*	X	✓	X	X
5807	*Arthrinium sp.*	X	✓	✓	X
5828	cf. *Leotiomycetes sp.*	X	X	✓	X

Table 33: Summarized results for the extracts, gained after lyophilization of strains, cultivated on Rice and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	Ec	Bv	Sc	Fo
5490				
5491				
5495				
5626				



Note: 5,5 mm Ø blank disks were used

4.3 Determination of Minimum Inhibitory Concentration (MIC) of the fungal secondary metabolites

In the following points the results of the MIC experiments, tested with strain extracts, that were cultivated on four different media and tested against the four model organisms in 96 deep well microplates. Each figure shows four graphs for four model organisms, with measured OD at 600 nm on y-axis and antimicrobial metabolite concentration (1024, 512, 256, 128, 64, 32, 18, 8, 4, 2 µg/mL) on x-axis. In the shown graphs, data lines for the measured time after 18h, 24h for both bacteria, 48h, 72h for yeast and 72 h for filamentous fungi are presented in different colours.

A shift in the minimum inhibitory concentration over time against one of the four model organisms could have several reasons, one could be the degradation of antimicrobial compounds over time or, that the antimicrobial metabolites evaporated during the cultivation time.

4.3.1 *Trichothecium* sp. (TUCIM 5490)

In Figure 9 results for the strain extract of *Trichothecium sp.* (TUCIM 5490) cultivated on rice medium are shown. An inhibition concentration for this strain can be tested with an antimicrobial compound concentration of 64 µg/mL against *B. velezensis* after 18/24 h. Additionally, to the inhibition against *B. velezensis*, an inhibitory concentration against *S. cerevisiae* with a concentration of 512 µg/mL over the measuring time, could be observed. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,2% and not shown.

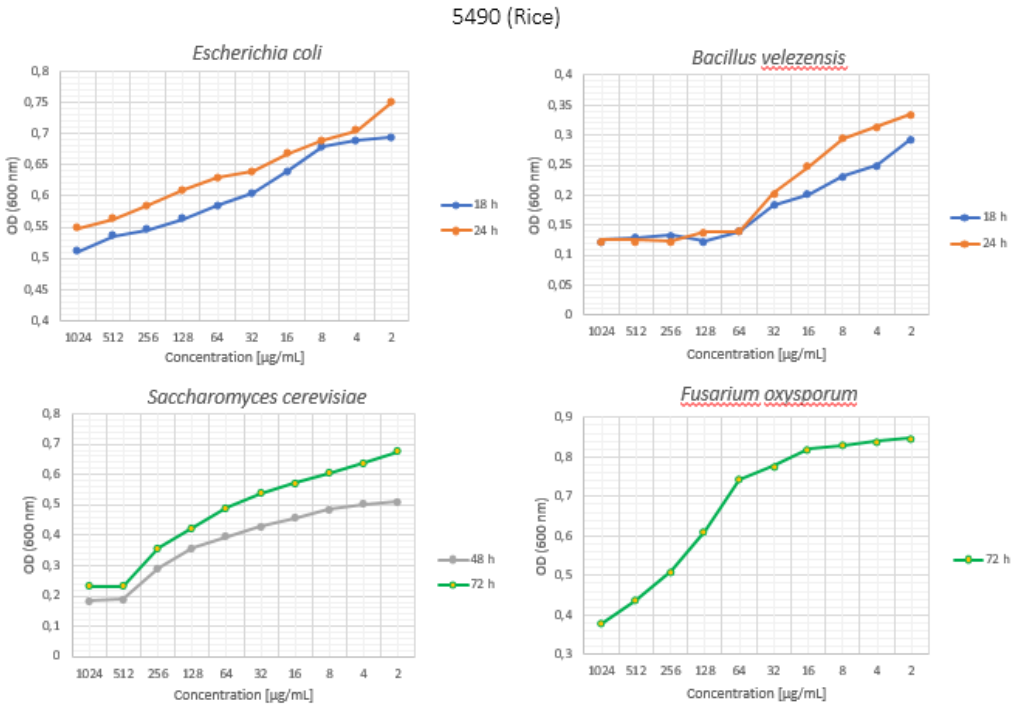


Figure 9: MIC experiment for the strain extract 5490, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.2 cf. *Nemania* sp. (TUCIM 5646)

Also, with strain extract 5646 (see Figure 10), cultivated on rice, an inhibition concentration against *B. velezensis* can be measured, with an extract concentration of 128 µg/mL (at 18 h), 256 µg/mL (at 24h). The mean value of two repeats is plotted and the calculated SD is below 0,3% and not shown.

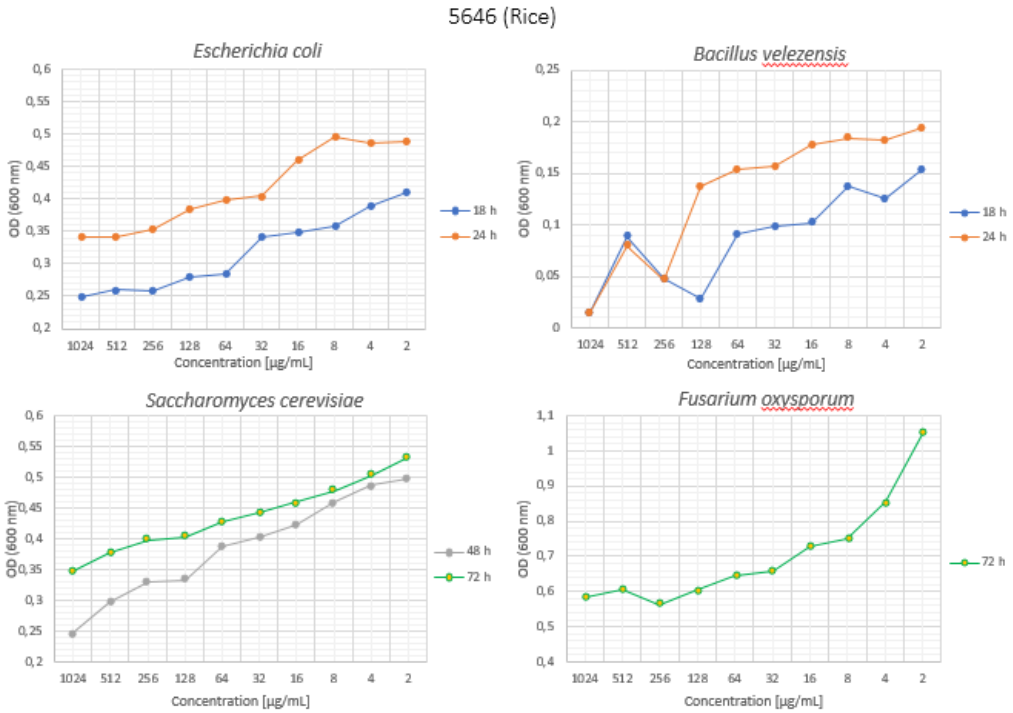


Figure 10: MIC experiment for the strain extract 5646, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.3 *Arthrinium* sp. (TUCIM 5807)

Figure 11 shows the MIC at 1024 µg/mL for strain 5807 against *B. velezensis* over the measured time of 24 h, after cultivation and extraction on Rice medium. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,1% and not shown.

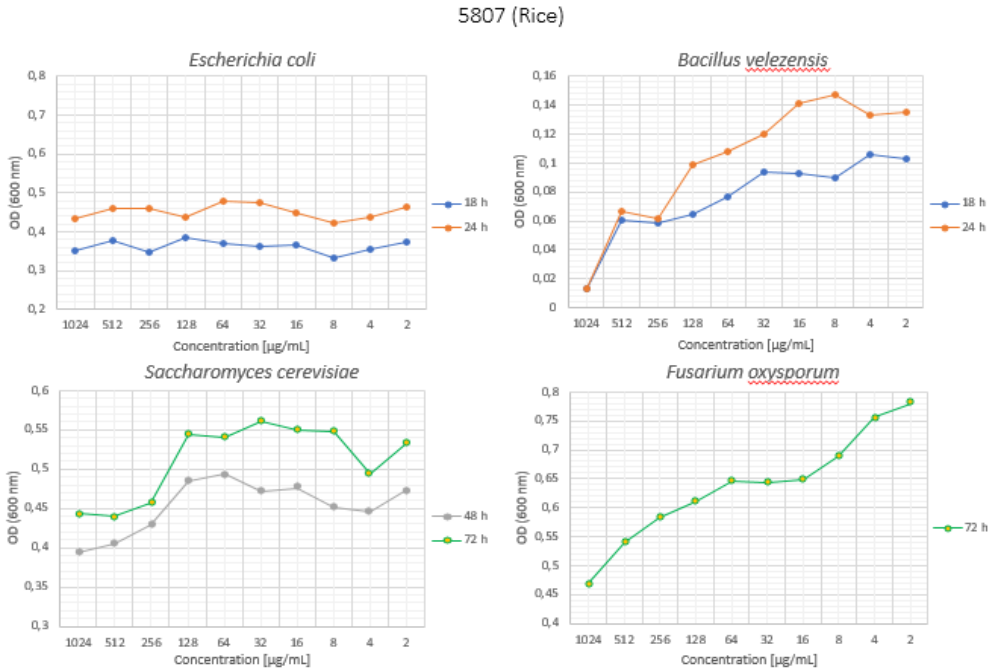


Figure 11: MIC experiment for the strain extract 5807, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.4 cf. *Paracremonium* sp. (TUCIM 5491)

In the next Figure 12, an inhibitory concentration of 64 µg/mL (24 h) against *B. velezensis* for strain 5491, cultivated on Rice medium, is shown. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,1% and not shown.

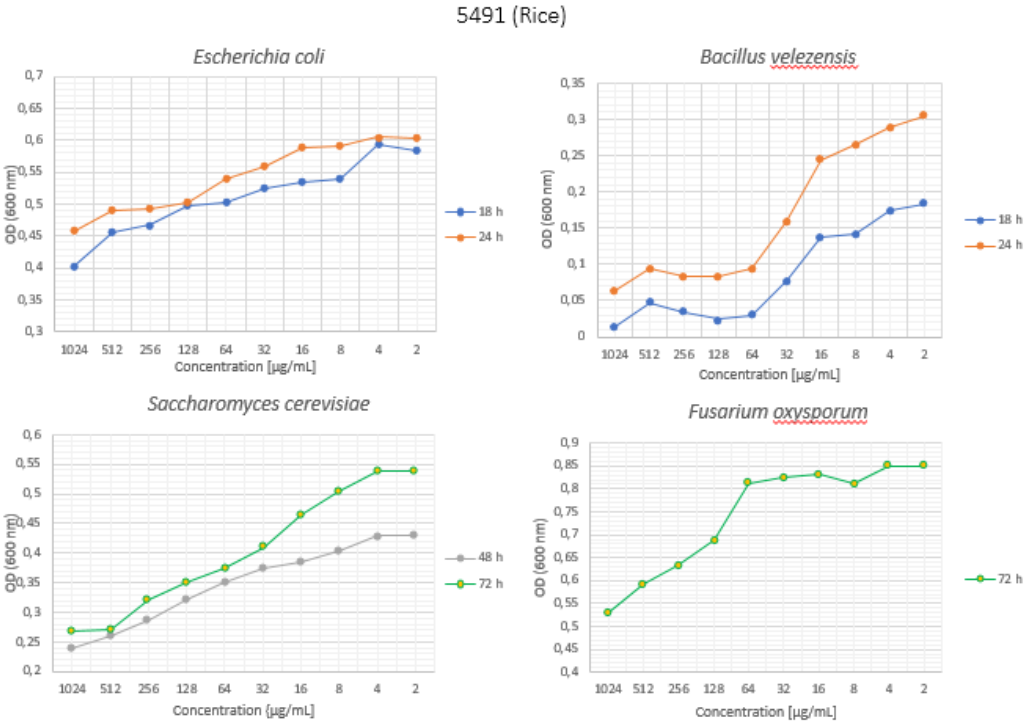


Figure 12: MIC experiment for the strain extract 5491, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.5 *Xylaria* sp. (TUCIM 5712)

The MIC of strain 5712 is 64 µg/mL (18/24 h), cultivated on Rice medium and 256 to 512 µg/mL (18/24 h), after cultivation on Malt Ex medium, against *B. velezensis* (see Figure 13-Figure 14). The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,3% and not shown.

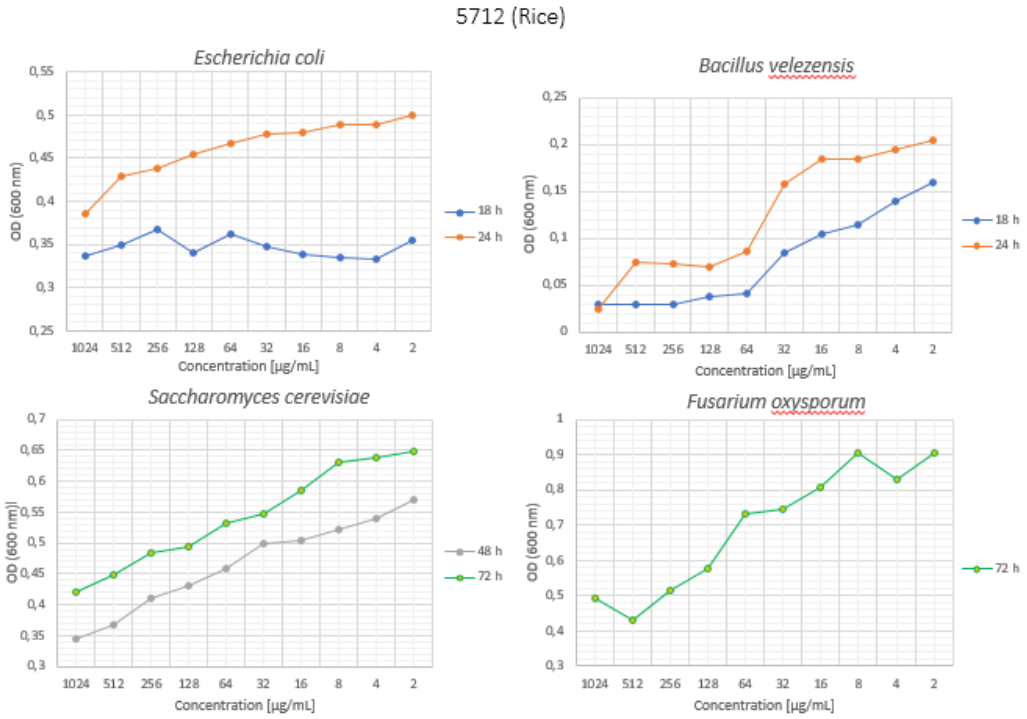


Figure 13: MIC experiment for the strain extract 5712, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

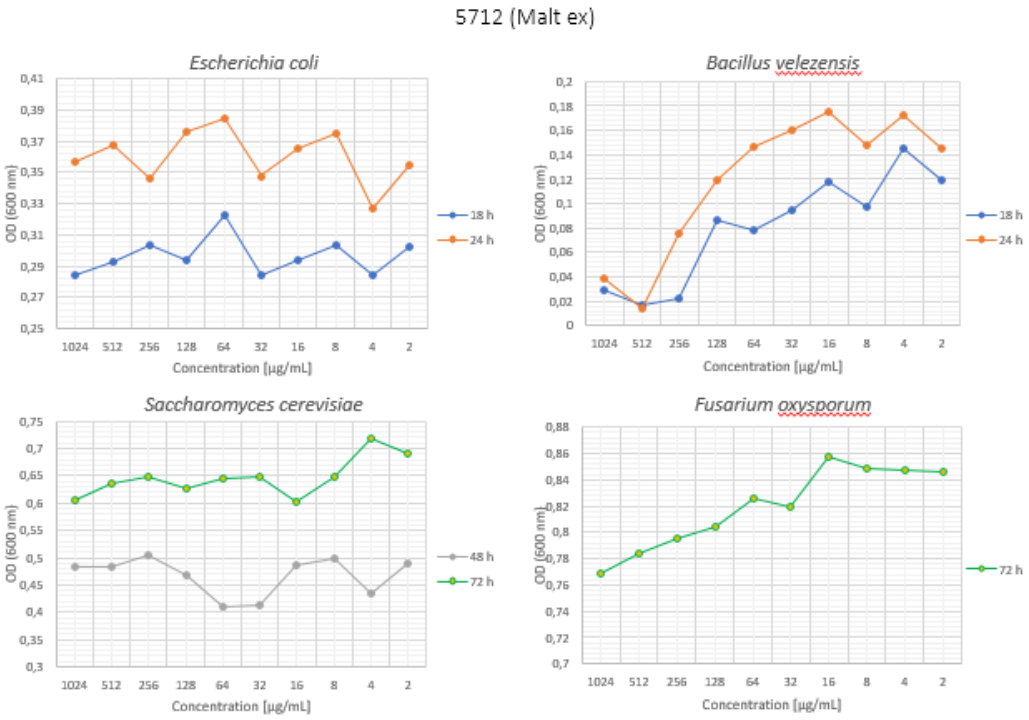


Figure 14: MIC experiment for the strain extract 5712, cultivated on Malt Ex and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.6 *Penicillium* rudallense (TUCIM 5767)

The minimum inhibitory concentration for this strain, cultivated on Rice, is shown in Figure 15, which could be tested against *B. velezensis* at a concentration of 32 µg/mL (18/24 h). The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,1% and not shown.

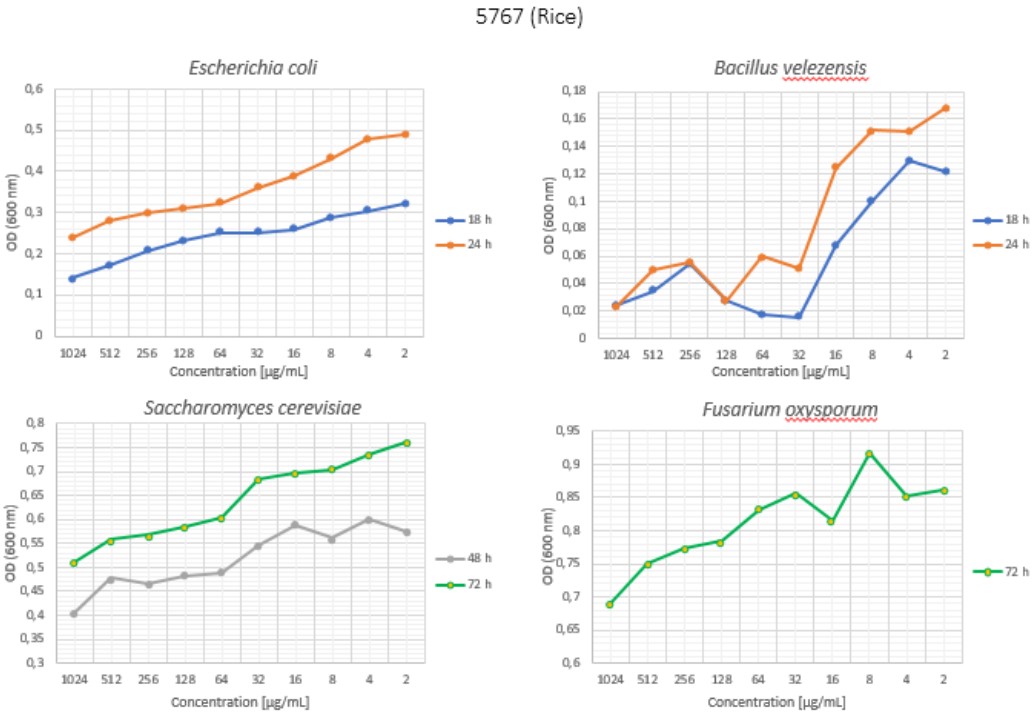


Figure 15: MIC experiment for the strain extract 5767, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.7 *P. expansum* (TUCIM 5626)

1024 µg/mL was the tested MIC for strain extract 5626 against *B. velezensis*, cultivated on Dox which can be seen in Figure 16. The same extract shows, after cultivation on Malt Ex medium, a MIC of 128 (18h) to 256 µg/mL (24 h) against *B. velezensis*, and against *E. coli* 128 (18 h) to 512 (24 h) µg/mL (see Figure 17). This could also be tested, when this strain extract was cultivated on PDA, which shows a MIC between 64 µg/mL (18 h) to 128 µg/mL (24 h) against *E. coli* and against *B. velezensis*, it varied between 32 µg/mL (18 h) to 64 µg/mL (24 h)18/24 h) (see Figure 18). The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,2% and not shown.

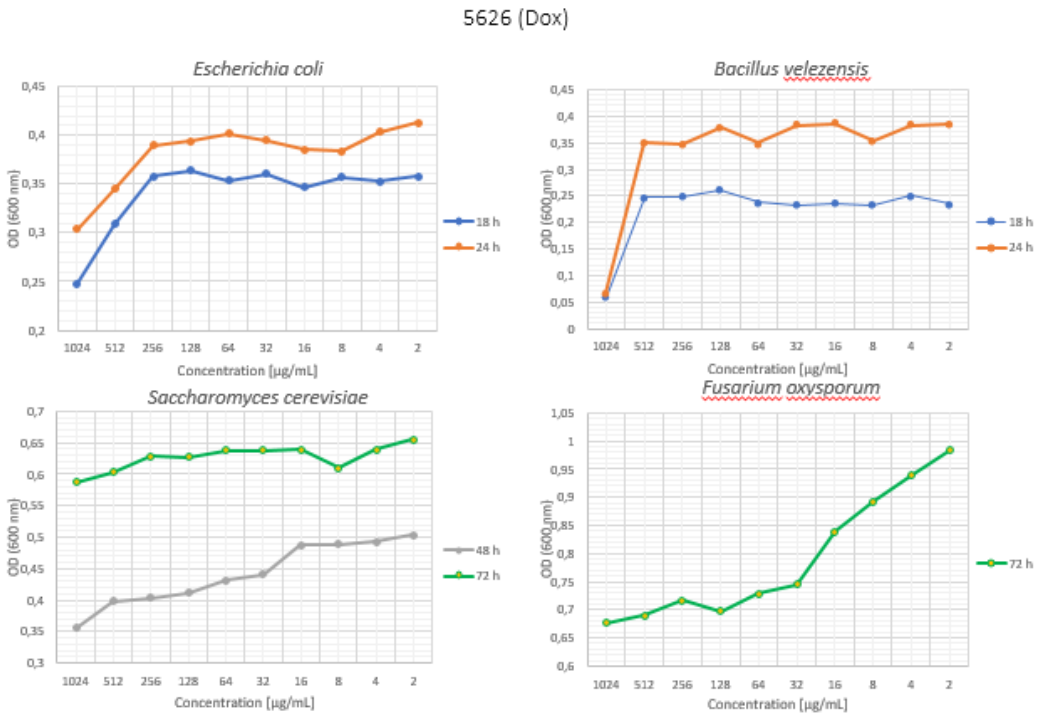


Figure 16: MIC experiment for the strain extract 5626, cultivated on Dox and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

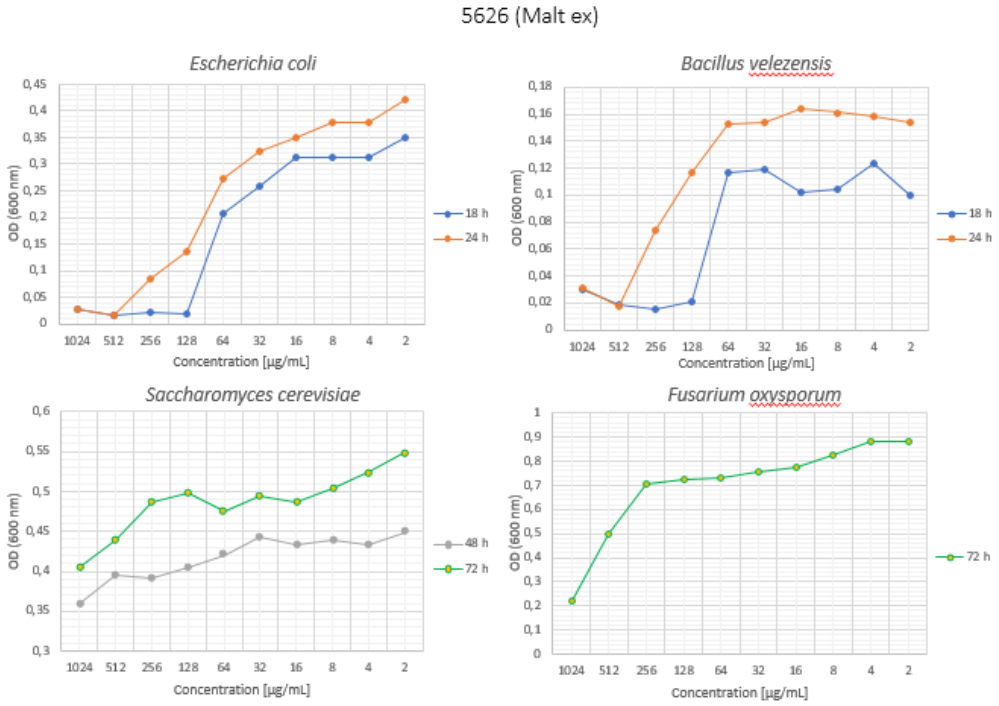


Figure 17: MIC experiment for the strain extract 5626, cultivated on Malt Ex and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

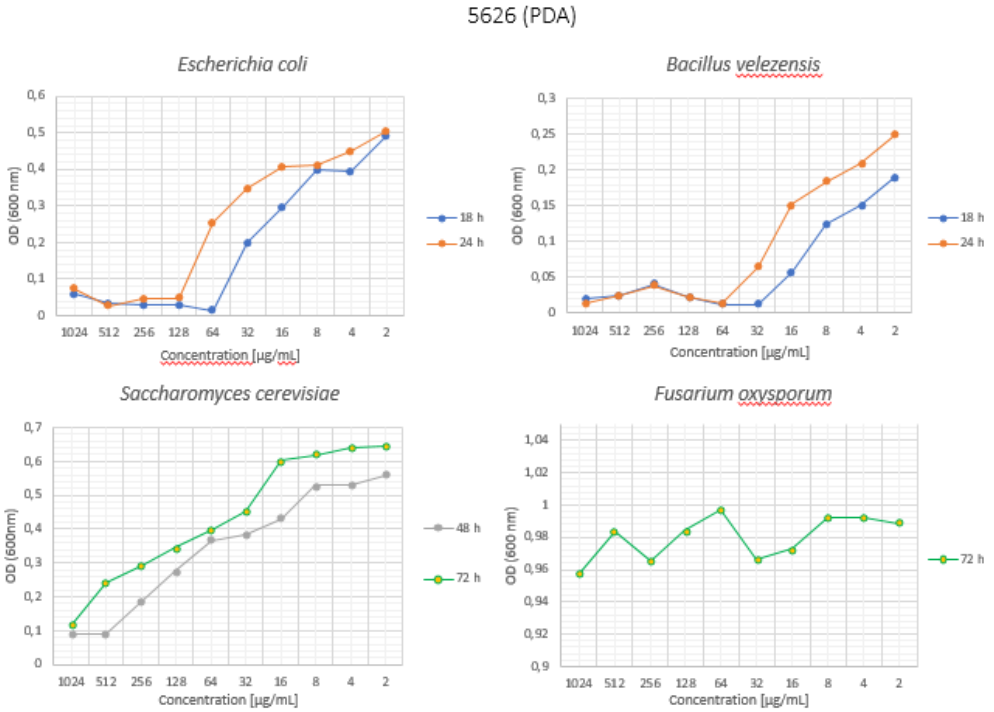


Figure 18: MIC experiment for the strain extract 5626, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.8 *Ovicillium* sp. (TUCIM 5628)

The MIC for this strain, cultivated on PDA, didn´t change over the measured time of 24 h and was tested against B. *velezensis* with a concentration of 128 µg/mL (see Figure 19). The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,3% and not shown.

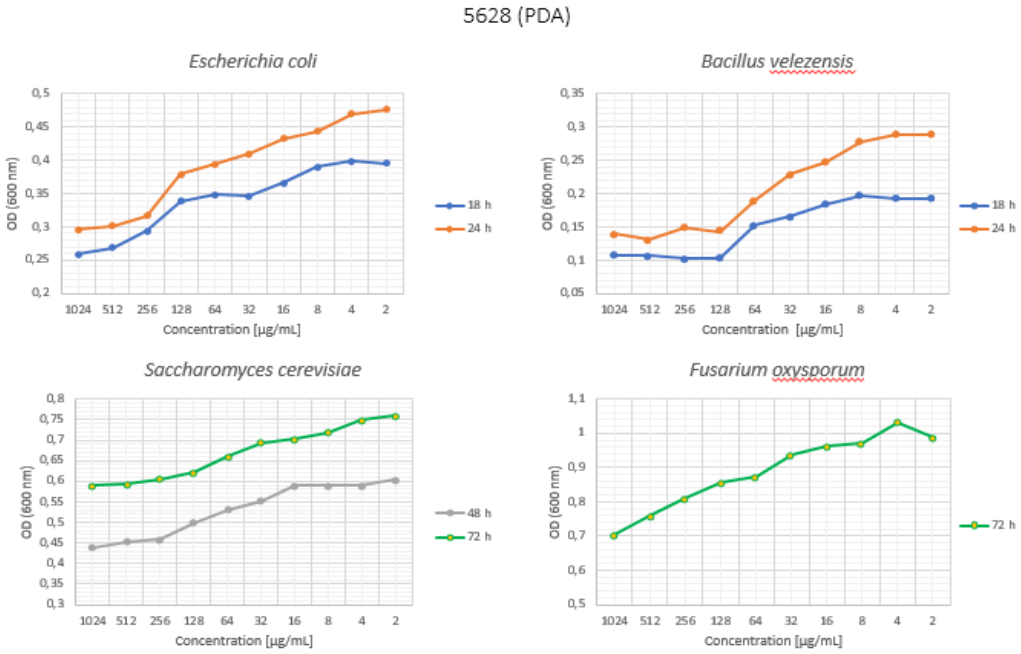


Figure 19: MIC experiment for the strain extract 5628, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F.oxysporum*

4.3.9 cf. *Leotiomycetes* sp. (TUCIM 5828)

256 µg/mL was the tested MIC for this strain extract against *B. velezensis* ,after cultivation on PDA, over the measured time slot, which can be seen in Figure 20. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,2% and not shown.

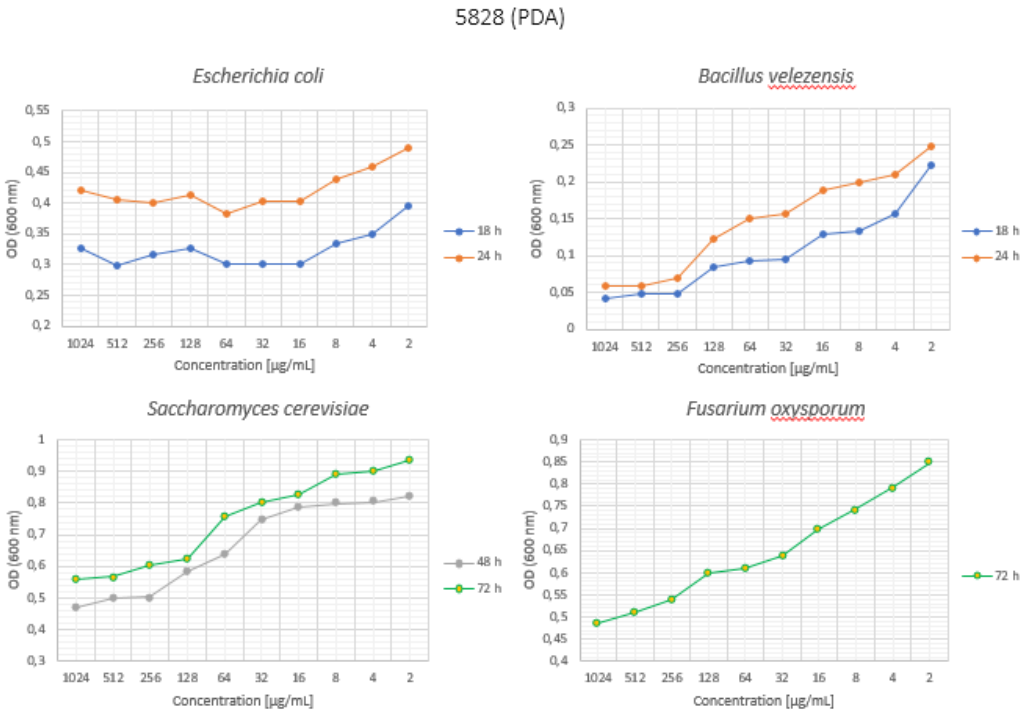


Figure 20: MIC experiment for the strain extract 5828, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.10 *A. rasikravindrii* (TUCIM 5773)

For this extract, a MIC of 32 µg/mL (18/24 h), cultivated on PDA, against *B. velezensis* could be measured over the measured time slot (see Figure 21). The same extract was also tested, after cultivation on Malt Ex against *B. velezensis* and showed a MIC of 128 µg/mL (18/24 h), which is shown in Figure 22. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,1% and not shown.

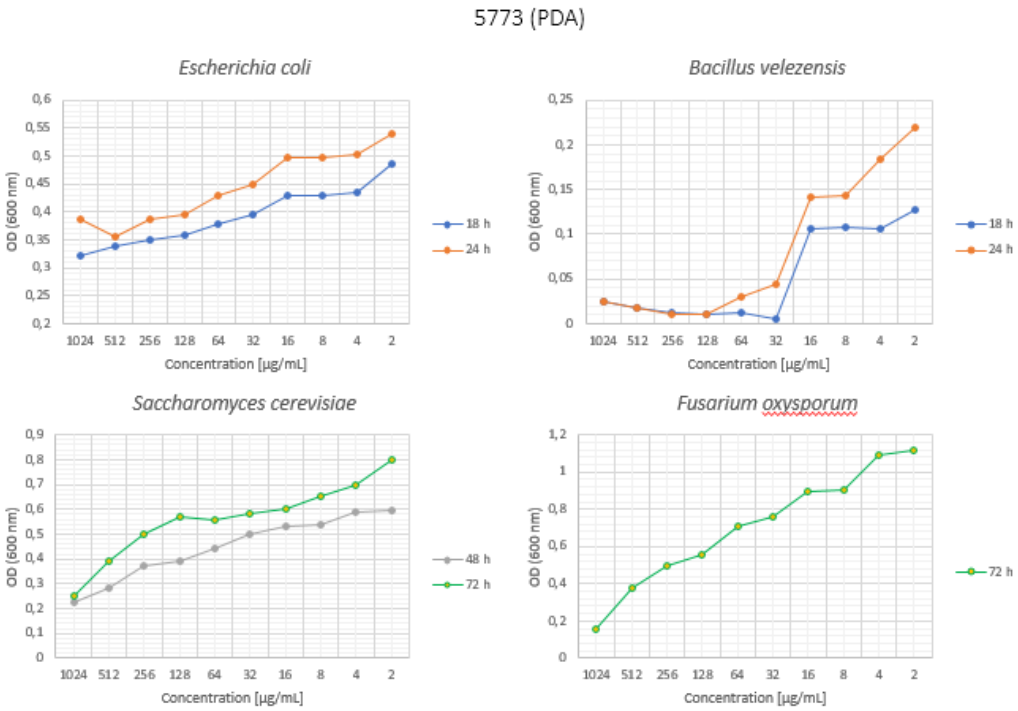


Figure 21: MIC experiment for the strain extract 5773, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

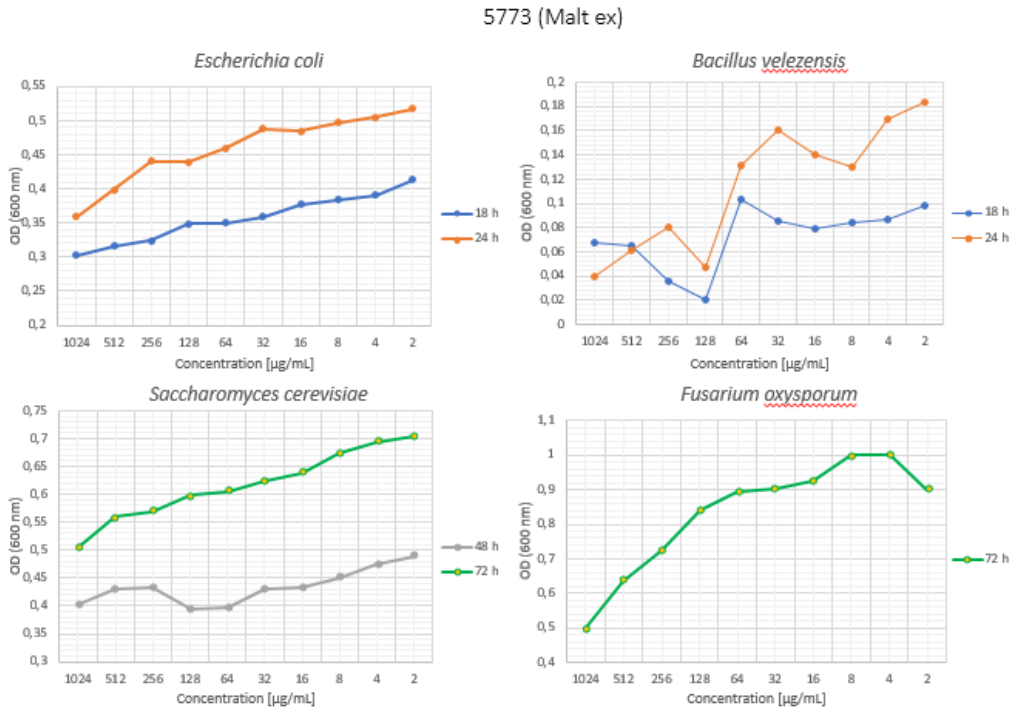


Figure 22: MIC experiment for the strain extract 5773, cultivated on Malt Ex and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.11 *Lasidioplodia* sp. (TUCIM 6037)

The strain extract showed, after cultivation on Malt Ex, a MIC of 256 µg/mL (18/24 h) against *B. velezensis* in Figure 23. The mean value of two repeats is plotted and the calculated standard deviation (SD) is below 0,3% and not shown.

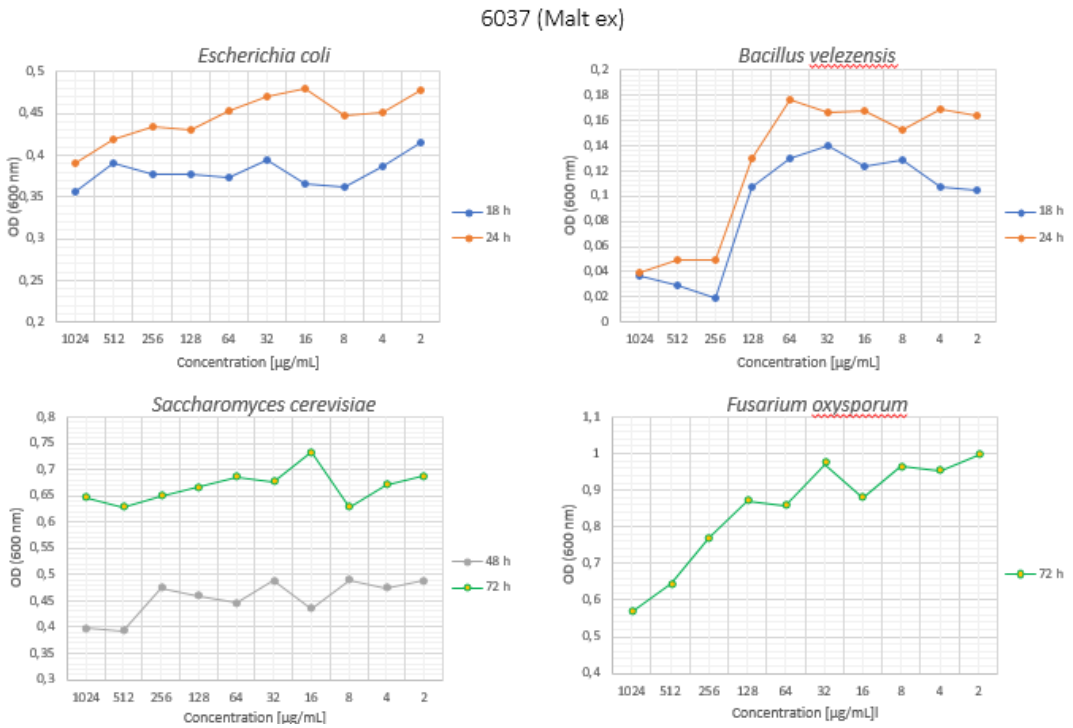


Figure 23: MIC experiment for the strain extract 6037, cultivated on Malt Ex and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

4.3.12 Summary of Minimum Inhibitory concentration (MIC) tested over four nutritional conditions

In Table 34 the summarized results of the MIC experiment are compared with the strain extracts, that showed in 4.2 an antimicrobial activity against one of the four model organisms. As shown, the MIC for *E. coli* and *B. velezensis* was taken for comparison at 24 h, for *S. cerevisiae* at 48 h and for *F. oxysporum* at 72 h. When the antimicrobial activity tested before on blank filter papers could be confirmed by measuring a minimum inhibitory concentration in the 96 deep well microplates, the results are labelled in green colours, if not, a red box represent the disagreement. The results, measured with strain extracts based on Malt Ex and Dox mediums, showed a minimum inhibitory concentration for all antimicrobial metabolite producers, that were tested before on filter papers. Strain extracts based on Rice and PDA media showed for several strains a MIC, but also some strains couldn´t be tested for their MIC against one model organism.

Conspicuous is strain 5828, which shows antimicrobial activity against S. cerevisiae, when tested on filter papers in both media(Rice and PDA), but no MIC could be tested in the 96 deep well microplates. Also, for strain 5495, 5801 on Rice and 5767, 5779, 5789 on PDA, no minimum inhibitory activity could be measured, although an antimicrobial activity was tested on blank filter papers. This could be caused by the fact, that the minimum inhibitory concentration is higher, than the measured concentration region. Interestingly, strain 5626 on Rice, which showed in all tested media on filter papers an inhibitory effect against least on model organisms, showed a measurable MIC in PDA, Malt Ex and Dox medium, but not in Rice medium. Overall, the measured minimum inhibition concentrations were the lowest, when the strain extracts were taken from the Rice and PDA mediums. Strains that showed no MIC are presented in the appendix at the end of the thesis.

Table 34: Summary of the MIC experiment tested against *E. coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc) and *F. oxysporum* (Fo)
(Green labelled cells confirmed a positive result in both used methods (MIC test compared to filter paper test), Red labelled cells show a mismatch of both methods)

TUCIM	Identification	Rice				Malt Ex				PDA				Dox			
		Ec [µg/mL]	Bv [µg/mL]	Sc [µg/mL]	Fo [µg/mL]	Ec [µg/mL]	Bv [µg/mL]	Sc [µg/mL]	Fo [µg/mL]	Ec [µg/mL]	Bv [µg/mL]	Sc [µg/mL]	Fo [µg/mL]	Ec [µg/mL]	Bv [µg/mL]	Sc [µg/mL]	Fo [µg/mL]
5490	*Trichothecium* sp.	> 1024	64	512	> 1024	-	-	-	-	> 1024	> 1024	> 1024	> 1024	-	-	-	-
5646	cf. *Nemania* sp.	> 1024	256	> 1024	> 1024	-	-	-	-	-	-	-	-	-	-	-	-
5807	*Arthrinium* sp.	> 1024	1024	> 1024	> 1024	-	-	-	-	> 1024	> 1024	> 1024	> 1024	-	-	-	-
5491	cf. *Paracremonium* sp.	> 1024	64	> 1024	> 1024	-	-	-	-	-	-	-	-	-	-	-	-
5712	*Xylaria* sp.	> 1024	64	> 1024	> 1024	> 1024	512	> 1024	> 1024	-	-	-	-	> 1024	> 1024	> 1024	> 1024
5767	*Penicillium rudallense*	> 1024	32	> 1024	> 1024	-	-	-	-	-	-	-	-	-	-	-	-
5626	*P. expansum*	> 1024	> 1024	> 1024	> 1024	512	256	> 1024	> 1024	128	64	> 1024	> 1024	> 1024	1024	> 1024	> 1024
5628	*Ovicillium* sp.	-	-	-	-	> 1024	> 1024	> 1024	> 1024	> 1024	128	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024
5828	cf. *Leotiomycetes* sp.	> 1024	> 1024	> 1024	> 1024	-	-	-	-	> 1024	256	> 1024	> 1024	-	-	-	-
5773	*A. rasikravindrii*	-	-	-	-	-	-	-	-	> 1024	32	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024
6037	*Lasidioplodia* sp.	-	-	-	-	> 1024	256	> 1024	> 1024	-	-	-	-	-	-	-	-
5495	*Pestalotiopsis* sp.	> 1024	> 1024	> 1024	> 1024	-	-	-	-								

4.4 Test ability to produce secondary metabolites under stress condition

4.4.1 Co-cultures

In the next few tables the results for the confrontation experiments are shown, where each table is ordered with the strains, that were confronted, in the top line against the strains in the left column. The results for each cell are presented with four number, e.g. 0/1/0/1, which resembles for *E. coli/B. velezensisv/S. cerevisiae/F. oxysporum*. The number 1 stands for a positive outcome, which result due to the inhibition of the model organism and the appearance of an inhibition halo, otherwise stands the number 0 for no reaction. Some numbers are also labelled with a question mark, because the outcome could also result of the growth bacteria.
Plugs for this experiment, where always taken from the point of contact of two confronting strains, to see, if they induce each other to produce antimicrobial metabolites or cancel each other out. To see, if the strains on their own are positive on the testing media, check the results written in point 4.1.1.

4.4.1.1 PDA

In Table 35 strains, which were tested on PDA and had shown positive results, were tested against each other. Additionally, also strains, that were negatively tested on PDA, where taken for this experiment, because, they could induce the production of antimicrobial metabolites. For strain:

5807 -> 5807 against himself hasn´t any effect of producing bioactive metabolites, against another testing strain, except for *B. velezensis*.

5830 -> Showed no activity on its own against all four testing strains, but shows now activity against *B. velezensis*, after confronting with strain 5789 and 6183. Those two strains had also shown no bioactivity against *B. velezensis* on their own, so by confronting them with strain 5830, an inducing effect can be accomplished.

5711 -> No reaction towards the four model organisms.

5767 -> Shows actually on PDA no bioactivity. Inhibitory effects against *B. velezensis* can be achieved, when it is confronted with strain 5801, 5635, 5806.

5789 -> Exhibit positive reactions against *B. velezensis*, after confronting with strain 5830, 5786 and 5773. Additionally, when its confronted with strain 5773, an antimicrobial activity towards *E. coli* and *S. cerevisiae* could be checked.

5779 -> No reaction towards the four model organisms.

5801 -> With strain 5767 and 5773 an inhibitory reaction towards *B. velezensis* can be seen.

5492 -> This strain shows bioactivity against *E. coli*, when its confronted with strain 5773.

5490 -> Same as for strain 5492, this strain shows positive activity against *E. coli* and *B. velezensis*, when its confronted with strain 5773.

5629 -> Shows a reaction against *B. velezensis* when its towards strain 5773.

5635 -> Has actually no bioactivity on its own, but, when its confronted with strain 5830, 5767, 5789, 5806 and 5773 the bioactivity towards *B. velezensis* must be induced somehow.

6183 -> Shows against 5830 a bioactivity versus *B. velezensis*, although, both strains were not bioactive on their own.

5786 -> Also, this strain shows no bioactive compounds against all four testing strains on its own, but after confronting with strain 5767, 5773 the bioactivity is induced towards *B. velezensis*.

5806 -> Bioactivity against *B. velezensis* after confronting with strain 5767, 5635 and 5773 can be seen. Additionally, to that, for the confrontation towards strain 5773 an antimicrobial activity versus *E. coli* could be tested.

5773 -> This strain shows the highest activity against all four testing strains, after the confrontation experiment, although, it has no bioactivity on its own at this media. Only versus 5807, 5711, 5767, 5779 and 6183 no antimicrobial activity could be tested, which indicates, that this strain can be induced easier than other strains or it has a strong inducing mechanism itself.

5628 -> Towards 5773, it gained, additionally to the ability to defend against *B. velezensis*, the capability to produce secondary metabolites against *E. coli*.

5828 -> For confronting versus strain 5773 it gains the ability to defend against *B. velezensis*.

Table 35: Summary of results for confrontation strains, grown on PDA and tested against *E.coli* (Ec)/*B. velezensis* (Bv)/*S. cerevisiae* (Sc)/*F. oxysporum* (Fo)

Strain	*5807*	*5830*	*5711*	*5767*	*5789*	*5779*	*5801*	*5492*	*5490*	*5629*	*5635*	*6183*	*5786*	*5806*	*5773*	*5628*	*5828*
5807	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5830	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/1	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5711	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5767	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0
5789	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	**1/1/1/0**	0/0/0/0	0/0/0/0
5779	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5801	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0
5492	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/0/0/0**	0/0/0/0	0/0/0/0
5490	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	0/0/0/0	0/0/0/0
5629	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/0/1/0**	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0
5635	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0
6183	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5786	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/0/1/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0
5806	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	0/0/0/0	0/0/0/0
5773	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/1/0**	0/0/0/0	**0/1/0/0**	**1/0/0/0**	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**0/1/0/0**
5628	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	0/0/0/0	0/0/0/0
5828	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0
Total Ec/Bv/Sc/Fo	**0/1/0/0**	**0/2/0/0**	**0/0/0/0**	**0/3/0/0**	**1/3/1/0**	**0/0/0/0**	**0/2/0/0**	**1/0/0/0**	**1/1/0/0**	**1/1/1/0**	**0/2/0/0**	**0/1/0/0**	**1/2/1/0**	**1/3/0/0**	**5/9/1/0**	**1/1/0/0**	**0/1/0/0**

4.4.1.2 YM

In Table 36 the results for the confrontation experiment on YM are shown. All strains, that were tested, showed antimicrobial activity against one of the four testing strains, when they were tested on their own. For strain:

5773 -> Against all other three strains (5628, 5626, 5712) a positive reaction towards *B. velezensis* could be tested, which indicates, that this strain has a strong inducing effect against other strains or can be induced easier than other ones. One thing should also be mentioned, that all tested strains showed positive reaction against *B. velezensis*, when they were tested all by their own.

5628 -> Also, with this strain, bioactivity towards *B. velezensis* could be accomplished, when its confronted versus 5773 and 5712, but the bioactivity is cancelled out, when its confronted against its own or strain 5626. This could probably be, when the bioactive compounds were based on a specific structure and the confronting partner produce enzymes, which degrade this structure (e.g. proteins will be degraded by proteases).

5626 -> Shows only activity against *B. velezensis*, when its confronted towards strain 5773. With the other strains, the ability to defend against *B. velezensis* is lost, which also can be, as written above, due to the degradation of the bioactive compounds.

5712 -> This strain shows, like 5773, inhibitory effects against *B. velezensis*, when its confronted versus all other strains. That could probably be, because the bioactive compounds, which strain 5712 produce, were based on a molecular structure, that can´t be degraded so easily. Another possible reason, why it shows a strong response to *B. velezensis*, when its confronted against the other strains, could be, that it has a strong inducing effect.

Table 36: Summary of results for confrontation strains, grown on YM and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5773*	*5628*	*5626*	*5712*
5773	**0/1/0/0**	**0/1?/0/0**	**0/1/0/0**	**0/1/0/0**
5628	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**
5626	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0
5712	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**
Total Ec/Bv/Sc/Fo	**0/4/0/0**	**0/2/0/0**	**0/1/0/0**	**0/3/0/0**

4.4.1.3 Dox

All results, for the confrontation experiment on Dox are presented in Table 37. For strain:

5490 -> Shows no inhibitory activity towards one of the four model organisms, when its confronted towards itself. Confronted against 5495, 5712, 5628, 5808, 5491, 5773 results in an inhibitory reaction against *B. velezensis*. After confronting towards strain 6986, an antimicrobial activity against *E. coli* can be tested, which is remarkable, because 5490 shows no activity and strain 6986 activity versus *B. velezensis*. So here, a shift of the antimicrobial activity from Grampositive to Gramnegative bacteria can be seen. Strain 5626 shows activity towards *E. coli*, but the confrontation experiment towards 5490, results in no reaction versus one of the model organisms. So, strain 5490 must degrade the antimicrobial compounds or induce to switch off the production of secondary metabolites of strain 5626.

5626 -> Towards itself, a reaction versus *E. coli* can be checked. The same results can be tested, after confronting versus strain 5628, 6992. Towards strain 5490, 5495 and 5808 no reaction can be seen. Additionally, to an activity towards *E. coli*, after confrontation versus 5491 and 5773, the ability to inhibit the growth of *B. velezensis* can be tested. Confrontation versus 6986 and 5712, an activity to inhibit the growth towards *B. velezensis* can be seen, so the activity against *E. coli* is cancelled out.

5495 -> Shows activity to inhibit the growth of Bacillus velezensis, after confronated with 5490, 6986, 5712, 5808, 5491 and 5773. Focussed should be at two strains: 5490, which shows no antimicrobial activity versus the four model organisms, when confronted against itself and strain 5626, which produce antimicrobial compounds towards *E. coli*.

6986 -> With this strain, towards *B. velezensis* all strains show an activity to inhibit the growth, except for 5490. Again, confronted with strain 5490, shows another result, then confronted with itself.

5712 -> Shows, after confronted with all strains, an antimicrobial activity versus *B. velezensis*. Additionally, to the activity towards *B. velezensis*, with strain 5808, a reaction against *E. coli* could be tested.

5628 -> Against strain 5495, 5628 and 6992, no reaction to inhibit the growth can be tested. This isn´t very unusual, because they show no reaction, when they were confronted towards itself. Confronted with strain 5626, 6986, 5712, 5808, 5491 and 5773 a reaction towards *E. coli* or *B. velezensis* can be tested, so this strain (5628) doesn´t change the antimicrobial metabolite production of the confronted strains. But, again, confrontation against strain 5490, shows another picture, by producing metabolites to inhibit the growth of *B. velezensis*.

5808 -> Except versus 5626, against all other strains an antimicrobial activity towards *B. velezensis* can be tested. Additionally, to that, after confronted towards 5712, an inhibitory activity versus *E. coli* can be checked.

5491 -> Shows towards all other strains an activity to stop the growth of *B. velezensis*. With strain 5626, also an activity to inhibit the growth of *E. coli* can be achieved.

5773 -> Also, when this strain is confronted with the other strains, an inhibitory activity versus *B. velezensis* can be tested. Confrontation with strain 5626 shows additionally to that an antimicrobial activity towards *E. coli*.

6992 -> Which shows no activity, when its confronted towards itself, shows inhibitory activity after confrontation with strain 6986, 5712, 5808, 5491 and 5773 versus *B. velezensis*. When this strain is confronted with strain 5626, it´s adapting the ability to produce antimicrobial compounds towards *E. coli*.

Table 37: Summary of results for confrontation strains, grown on Dox and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5490*	*5626*	*5495*	*6986*	*5712*	*5628*	*5808*	*5491*	*5773*	*6992*
5490	0/0/0/0	0/0/0/0	**0/1/0/0**	**1/0/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0
5626	0/0/0/0	**1/0/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**1/0/0/0**	0/0/0/0	**1/1/0/0**	**1/1/0/0**	**1/0/0/0**
5495	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0
6986	**1/0/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5712	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5628	**0/1/0/0**	**1/0/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0
5808	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5491	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5773	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
6992	0/0/0/0	**1/0/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0
Total Ec/Bv/Sc/Fo	**1/6/0/0**	**5/4/0/0**	**0/6/0/0**	**1/9/0/0**	**1/10/0/0**	**1/6/0/0**	**1/9/0/0**	**1/10/0/0**	**1/10/0/0**	**1/5/0/0**

4.4.1.4 Malt Ex

In Table 38 the results for the confrontation experiment on Malt Ex are presented. All strains, that were tested, except of strain 5828 and 5779, showed positive reaction against one of the four testing strains, when they were tested on their own. For strain:

5626 -> Shows antimicrobial activity for *B. velezensis* after confrontation with strain 6037, 5773 and 5626. Additionally, 5626 also has the capability to produce bioactive compounds against *E. coli*, when its confronted against itself. Confrontation with other strains has the effect, that 5626 lose the ability to produce compounds to inhibit the growth of *B. velezensis* and *E. coli*.

5712 -> Except for 5828, the other positive strains 5712 and 5628 produce bioactive metabolites against *B. velezensis*, when they were cultivated on Malt Ex on their own. So, the interesting part here, is the confrontation of 5712 towards 5828, which result in a positive reaction against *B. velezensis*. This implies, that 5828 can´t degrade produced antimicrobial compounds from strain 5712 and that's why, a positive test result towards *B. velezensis* can be seen.

5492 -> Only one strain, 5628, has the capability to produce bioactive compounds, when its confronted with strain 5492. A reason for this could be, that strain 5492 produce an enzyme mix, which degrades most of the produced antimicrobial compounds.

6037 -> Three positive results against *B. velezensis*, could be tested with this strain, after confrontation towards 5626, 6037, 5779. Strain 5626 and 6037 show positive results against *B. velezensis*, when they were tested on their own, so a positive result, after they confronted themselves, isn´t very unusual. For strain 5779, which has no ability of producing bioactive compounds, the positive test against *B. velezensis* indicates, that isn't producing enzymes, which can degrade the produced antimicrobial compounds of strain 5712.

5779 -> Actually, no bioactive metabolites are produced by this strain, but, when it will be confronted versus 6037, 5628 and 5773 a positive result towards *B. velezensis* can be accomplished. This implies, that 5779 produce against the other negative tested strains enzymes, which degrade their bioactive metabolites.

5828 -> Also this strain has no bioactive metabolite producing ability against the four testing strains. With strain 5712, 5628 and 5773, this strain gets the capability to inhibit the growth of *B. velezensis*, which is interesting, because, as mentioned, it has no bioactive producing ability, like strain 5779, against the four testing strains, but with strain 5628 and 5773, this ability can be gained through confrontation. So, strain 5779 and 5828 produce quite the same enzyme classes to degrade, from other strains, their antimicrobial compounds, because they show no effect, when they were confronted with five out of eight strains and only differ in one positive strain.

5628 -> Shows a positive result against *B. velezensis* after confronted towards six out of eight tested strains. This indicates, that the bioactive compounds produced by this strain are based on specific elements, because against strain 5492 it shows a positive reaction, but 5492 itself shows no reaction against all other strains.

5773 -> Same as for strain 5628, this strain shows a strong response towards *B. velezensis*, after confrontation against five out of eight strains.

Table 38: Summary of results for confrontation strains, grown on Malt Ex and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5626*	*5712*	*5492*	*6037*	*5779*	*5828*	*5628*	*5773*
5626	**1/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**
5712	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0
5492	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0
6037	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0
5779	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**
5828	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**
5628	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5773	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
Total Ec/Bv/Sc/Fo	**1/3/0/0**	**0/3/0/0**	**0/1/0/0**	**0/3/0/0**	**0/3/0/0**	**0/3/0/0**	**0/6/0/0**	**0/5/0/0**

4.4.1.5 YESD

In Table 39, the results for the confrontation experiments on YESD are summarized. All strains that were tested, except for 5712, 5626 and 5801, show an antimicrobial activity towards one of the model organisms. For strain:

5712 -> Shows no inhibitory activity towards the four model organisms, when its confronted against itself. But confrontation towards 5626, 6986, 5808, 5773 and 5801 induce the production of secondary metabolites to inhibit the growth of *B. velezensis* and for 5808 and 5773 additionally versus *E. coli*.

5626 -> Towards 5712, 6986, 5773 and 5801 an antimicrobial activity versus *B. velezensis* can be achieved. It´s interesting, that confrontation against 5712 result in an inhibitory effect at the growth of *B. velezensis*, when both strain produce no antimicrobial metabolites against this organism after confronted with itself.

5628 -> Results show, that after confrontation with the other strains, except of 5626, this strain produce metabolite to stop the growth of *Bacillus velezensis*. That's not very unusual, because as written before, strain 5626 produce no antimicrobial metabolites against the four testing strains and the remaining strains produce metabolites towards *B. velezensis*.

6986 -> This strain shows confronted against all other strains an inhibitory activity towards *B. velezensis*. So, strain 5712, 5626 and 5801, which show actually no reaction, when they were confronted against itself, produce no compounds to degrade the produced metabolites of strain 6986.

5808 -> Also this strain produce, after confronted with all other strains, except for strain 5626, antimicrobial metabolites versus *B. velezensis*, but when its confronted towards strain 5712, it is showing additionally, an activity towards *E. coli*.

5773 -> Shows towards all other strains, except towards strain 5712, the same reaction by producing antimicrobial compounds to inhibit the growth of *B. velezensis*. For 5712, as written also for strain 5808, additionally an activity against *E. coli* could be tested.

5801 -> Shows no activity, when its confronted towards itself, but adapts the ability to produce antimicrobial compounds versus *B. velezensis*, after confrontation with the reaming strains. Also, with strain 5712 and 5626, which show no activity, after confrontation with itself, a reaction to stop the growth of Bacillus velezensis, can be achieved.

Table 39: Summary of results for confrontation strains, grown on YESD and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5712*	*5626*	*5628*	*6986*	*5808*	*5773*	*5801*
5712	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**1/1/0/0**	**0/1/0/0**
5626	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**
5628	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
6986	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5808	**1/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5773	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5801	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0
Total Ec/Bv/Sc/Fo	**2/5/0/0**	**0/4/0/0**	**0/6/0/0**	**0/7/0/0**	**1/6/0/0**	**1/7/0/0**	**0/6/0/0**

4.4.1.6 PYG

In Table 40 the results for the confrontation experiment on PYG are presented. All strains, that were tested, except for 5767, showed positive reaction against one of the four testing strains, when they were tested on their own. For strain:

5628 -> The bioactivity against *B. velezensis* wasn´t changed after the confrontation against 5773, but was erased, when it was confronted towards 5767.

5773 -> The same happed with strain 5773, when it was confronted with 5626 and 5767.

5767 -> Confrontations against this strain, result in no reaction towards the four model organisms .

Table 40: Summary of results for confrontation strains, grown on PYG and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/Fusarium*

Strain	*5628*	*5773*	*5767*
5628	**0/1/0/0**	**0/1/0/0**	0/0/0/0
5773	**0/1/0/0**	**0/1/0/0**	0/0/0/0
5767	0/0/0/0	0/0/0/0	0/0/0/0
Total Ec/Bv/Sc/Fo	**0/2/0/0**	**0/2/0/0**	**0/0/0/0**

4.4.1.7 YES

Summarized results for medium YES are shown in Table 41. All strains, except for 5711, show an inhibitory activity towards one of the four testing strains. For strain:

5491 -> Shows activity towards *B. velezensis*, when its confronted with itself. Also, after confronted with strain 6986, 5773 and 5808 result in an activity versus *B. velezensis*. Against strain 5711, no activity can be seen.

5711 -> No activity can be checked, after confronted with itself. Only after the confrontation with strain 6986 and 5773 an antimicrobial activity towards *B. velezensis* can be tested. So, confrontation with strain 5491 and 5808 results in no inhibitory activity versus the four model organisms, which indicates, that they are producing enzymes to degrade the produced antimicrobial compounds.

6986 -> This strain, shows, when its confronted with strain 5491, 6986 and 5808 an antimicrobial activity versus *B. velezensis*. Against 5711 no inhibitory activity towards the four model organisms can be tested. An inhibitory activity against two model organisms can be achieved, when this strain is confronted towards strain 5773.

5808 -> Shows against *B. velezensis* inhibitory activity, when its confronted versus strain 5491, 6986 and itself. Here, strain 5711 and 5773 could produce enzymes to degrade the antimicrobial metabolites produce by strain 5808, after confrontation.

Table 41: Summary of results for confrontation strains, grown on YES and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5491*	*5711*	*6986*	*5773*	*5808*
5491	**0/1/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5711	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	0/0/0/0
6986	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**
5773	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	0/0/0/0
5808	**0/1/0/0**	0/0/0/0	**0/1/0/0**	0/0/0/0	**0/1/0/0**
Total Ec/Bv/Sc/Fo	**0/4/0/0**	**0/2/0/0**	**1/4/0/0**	**1/4/0/0**	**0/3/0/0**

4.4.1.8 Oat Flour

In Table 42 the results for the confrontation experiment on Oat Flour are presented. All strains, that were tested, except of strain 6992, showed positive reaction against one of the four testing strains, when they were tested on their own. For strain:

5628 -> Shows a positive reaction against *B. velezensis*, after confrontation towards itself and strain 5712. Against 5626 no bioactivity can be seen, although both strains produce secondary metabolites versus *B. velezensis*, when they were cultivated separated. This implies, that both strains produce enzymes, which degrade the bioactive compounds of the other strain.

5712 -> The same as wrote above for strain 5628, also count for this strain.

5626 -> Towards 5628 and 5712 no reaction can be seen, but when its cultivated against itself or strain 6992 a positive reaction versus *E. coli* and *B. velezensis* results. When strain 5626 is cultivated alone, it produces secondary metabolites to inhibit the growth of *B. velezensis* and *E. coli*, but, after confronting with itself, it loses this ability against *B. velezensis*. Strain 6992 doesn´t affect the bioactive compounds of strain 5626, which why the result for strain 5626 itself and confronted towards 6992 is the same.

6992 -> Produces itself no bioactive compounds, as you can see in Table 21, but, when its confronted against strain 5626 it is adapting the bioactive compounds of this strain to inhibit the growth of *E. coli* and *B. velezensis*.

Table 42: Summary of results for confrontation strains, grown on Oat Flour and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	***5628***	***5712***	***5626***	***6992***
5628	**0/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0
5712	**0/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0
5626	0/0/0/0	0/0/0/0	**1/0/0/0**	**1/1/0/0**
6992	0/0/0/0	0/0/0/0	**1/1/0/0**	0/0/0/0
Total Ec/Bv/Sc/Fo	**0/2/0/0**	**0/2/0/0**	**1/1/0/0**	**1/1/0/0**

4.4.1.9 Rice

In Table 43 the results for the confrontation experiment on Rice are presented. All strains, that were tested, except of strain 5830, 5773 and 5807, showed positive reaction against one of the four testing strains, when they were tested on their own. For strain:

5646 -> No antimicrobial activity towards the four model organisms can be seen, after confronted with itself. But, when its confronted with strain 5626, 5773, 5628, 5807 and 5490 an activity to inhibit the growth of *B. velezensis* can be tested. Additionally, to that, against strain 5626 an inhibitory activity towards *E. coli* and for strain 5628, 5807 and 5490 an antimicrobial activity versus *S. cerevisiae* can be seen.

5626 -> Shows towards eight out of nine samples a positive results, which is nearly the same results, as for cultivating the strain alone. Only against strain 5801 and 5628 the bioactivity versus *B. velezensis* is gone and for the confrontation against strain 5773, no positive reaction could be tested. This implies, that the produced bioactive compounds were degraded by the confronted strain or the confronted strain secrets chemicals, that shut down the gen cluster, which is responsible for producing the bioactive compounds.

5830 -> Has no bioactive compounds, which are produced by the strain itself. So, the positive results must be related to the confronted strains, but since both other strains, which result in a positive reaction against *E. coli* and *B. velezensis*, are also no bioactive compound producer, an inducing effect must happen.

5773 -> Shows, confronted with strain 5646, 5830, 5628, 5807 and 5490 an antimicrobial activity against *B. velezensis*.

5801 -> Against itself, no inhibitory activity towards one of the four testing organisms can be checked. Confrontation versus strain 5628, 5807 and 5490 result in an antimicrobial activity against *B. velezensis*.

5628 -> With this strain, six out of eight positive results could be achieved. Towards strain 5491, which both have no bioactivity against *E. coli*, a bioactivity can be tested against *E. coli*. So, after the confrontation, one of the two strains must be triggered to the appearance of the other one, which result in producing bioactive compounds towards *E. coli*.

5807 -> This strain is the perfect sparring partner for other strains, because it shows towards all other strain bioactivity at least against one of the four testing strains. Some of the strains, like 5491 or 5801, gain or lose the ability to inhibit the growth of one testing strain and some of the confronted strains, like 5626 and 5628, were not affected by the appearance of this strain. As already written at strain 5830, also this strain is inducing the non-bioactive compound producer and itself. Towards strain 5626, an activity to inhibit the growth of *E. coli* can be tested.

5491 -> Shows no inhibitory activity, when its confronted against itself. But confrontation towards strain 5626, 5629 and 5807 result in an antimicrobial activity versus *E. coli* and *B. velezensis*. For strain 5626, its obvious, that strain 5491 is not switching off the production of antimicrobial metabolites or degrading the produced antimicrobial metabolites of strain 5626. But towards strain 5628 and 5807, which produce antimicrobial metabolites towards *B. velezensis*, additionally an activity to inhibit the growth of *E. coli* can be tested.

5490 -> Five out of eight samples are positive tested, when they were confronted with this strain. With strain 5626 it is adapting the bioactive compounds, which result in a positive reaction against *E. coli* and *B. velezensis*. With strain 5807 and 5773, that are non-bioactive metabolite producer, a positive reaction towards *B. velezensis* and *S. cerevisiae* could be tested, which is interesting, because strain 5490 alone shows only versus *B. velezensis* bioactivity.

Table 43: Summary of results for confrontation strains, grown on Rice and tested against *E.coli (Ec)/B. velezensis (Bv)/S. cerevisiae (Sc)/F. oxysporum (Fo)*

Strain	*5646*	*5626*	*5830*	*5773*	*5801*	*5628*	*5807*	*5491*	*5490*
5646	0/0/0/0	**1/1/0/0**	0/0/0/0	**0/1/0/0**	0/0/0/0	**0/1/1/0**	**0/1/1/0**	0/0/0/0	**0/1/1/0**
5626	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	0/0/0/0	**1/0/0/0**	**1/0/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**
5830	0/0/0/0	**1/1/0/0**	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	**1/1/0/0**	0/0/0/0	**0/1/0/0**
5773	**0/1/0/0**	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**
5801	0/0/0/0	**1/0/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/0/0**
5628	**0/1/1/0**	**1/0/0/0**	0/0/0/0	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	0/0/0/0
5807	**0/1/1/0**	**1/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/0/0**	**0/1/1/0**
5491	0/0/0/0	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0
5490	**0/1/1/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	0/0/0/0	**0/1/1/0**	0/0/0/0	0/0/0/0
Total Ec/Bv/Sc/Fo	**1/5/3/0**	**8/6/0/0**	**2/4/0/0**	**0/5/0/0**	**1/3/0/0**	**2/6/0/0**	**3/9/2/0**	**3/3/0/0**	**1/6/2/0**

4.4.2 Starvation

In the next three tables (see Table 44-Table 46), the results for the starvation experiments are presented. In the top line of the tables, the time points, where the cultivated strains were measured, as well as, in the second line the four testing strains against the first row of the selected strains for this experiment. The number 1 stands for a positive outcome, which result due to inhibit of the testing strain and the appearance of a halo, otherwise stands the number 0 for no reaction. All plates were cultivated under the same conditions (medium plates, 28°C) and measured at the same time. Only three media were used for the experiment, because they showed from previous tests, that they contain the highest number of antimicrobial metabolite producers for the 40 used fungi samples and were common media in the industry.

4.4.2.1 PDA

As shown in Table 44, three strains, that are showing bioactivity against *B. velezensis* could be tested. The differ can be seen, after comparing this table with Table 5, which result in three positive strains here out of five positive strain in the other table. Unfortunately, strain 5807 and 5786, which were tested positive in Table 5, were not tested here, so I can only analyse these strains here. Strain 5492, which was actually positive tested in Table 5, shows no bioactivity after two weeks towards *S. cerevisiae*. This could be, because the bioactive compounds against *S. cerevisiae* were already degraded and reused for other metabolites or the bioactive metabolites were not stable and were only produced during the growth of the fungus.

For strain 5628, the same result after two weeks could be achieved, but one week later, it loses the bioactivity against *B. velezensis*, which could be, because degradation and reuse for other important substances.

The bioactivity of strain 5629 against *B. velezensis* could be confirmed after two weeks, but it also loses the ability to inhibit the growth of *S. cerevisiae* . After three weeks, the capability to produce bioactive compounds against *B. velezensis* is also lost.

For strain 5773 bioactive compounds against *B. velezensis* after two weeks and against *E. coli* after three weeks, could be tested, but, as always, the strain plug was framed with a slimy layer of grown bacteria, so these results must be taken with cautions.

After six weeks of cultivation at 28°C, all strains show no antimicrobial activity against the four model organisms.

Table 44: Summary of results for several strains, grown on PDA and tested after certain timepoints against *E. coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc), F. oxysporum (Fo)*

	14 days				*21 days*				*42 days*			
Strain	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***
5490	0	0	0	0	0	0	0	0	0	0	0	0
5492	0	0	0	0	0	0	0	0	0	0	0	0
5628	0	**1**	0	0	0	0	0	0	0	0	0	0
5629	0	**1**	0	0	0	0	0	0	0	0	0	0
5635	0	0	0	0	0	0	0	0	0	0	0	0
5711	0	0	0	0	0	0	0	0	0	0	0	0
5767	0	0	0	0	0	0	0	0	0	0	0	0
5773	0	**1**	0	0	**1**	**1**	0	0	0	0	0	0
5779	0	0	0	0	0	0	0	0	0	0	0	0
5789	0	0	0	0	0	0	0	0	0	0	0	0
5806	0	0	0	0	0	0	0	0	0	0	0	0
5828	0	0	0	0	0	0	0	0	0	0	0	0
5830	0	0	0	0	0	0	0	0	0	0	0	0
6183	0	0	0	0	0	0	0	0	0	0	0	0

4.4.2.2 Dox

Table 45 presents the results of the starvation experiment for the Dox media. As exhibited, five strains, that are showing bioactivity against one of the four testing strains could be tested compared to Table 9, which result in eight positive strains. Strain 5491 shows over the measured time of six weeks the same bioactivity against *B. velezensis*.

For strain 5626, that should produce bioactive compounds against *E. coli* and *B. velezensis*, the outcome after two weeks, resulted in a positive reaction against *E. coli*. The ability to inhibit the growth of *E. coli* is lost, after three weeks of cultivation.

Less than two weeks of grow showed for strain 5628 and 5808 in Table 9 an inhibition of *B. velezensis*, which can´t be confirmed, after two weeks of growing. This implies, that the bioactive compounds were produced only in the early growing phase and were degraded later for new metabolites.

5712, which produce bioactive metabolites against *E. coli* and *B. velezensis*, when it was cultivated less than two weeks, shows after two weeks, only a bioactivity towards *B. velezensis*. The bioactivity against *B. velezensis* remain, also after three weeks of cultivation, but the activity towards *E. coli* wasn´t returning.

For strain 5773, also after three weeks of cultivation, the problem with the co-culture of bacteria cannot be eliminated. Although, after two weeks of cultivation, bioactivity against *E. coli* and *B. velezensis* could be tested, the activity drops down towards *B. velezensis*, after cultivation strain 5773 over three weeks.

Strain 6986 shows the same bioactivity over six weeks of cultivation against *B. velezensis*.

The last strain, 6992, which produce bioactive compounds against *E. coli* and *S. cerevisiae* , lose this ability within two weeks of cultivation and doesn´t gained it again after three weeks of cultivation.

Except of strain 5491 and 6986, all other strains lose their ability to inhibit the growth towards the four testing strains, after cultivation of six weeks.

Table 45: Summary of results for several strains, grown on Dox and tested after certain timepoints against *E.coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc), F. oxysporum (Fo)*

	14 days				*21 days*				*42 days*			
Strain	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***
5490	0	0	0	0	0	0	0	0	0	0	0	0
5491	0	**1**	0	0	0	**1**	0	0	0	**1**	0	0
5495	0	0	0	0	0	0	0	0	0	0	0	0
5626	**1**	0	0	0	0	0	0	0	0	0	0	0
5628	0	0	0	0	0	0	0	0	0	0	0	0
5712	0	**1**	0	0	0	**1**	0	0	0	0	0	0
5773	**1**	**1**	0	0	0	**1**	0	0	0	0	0	0
5808	0	0	0	0	0	0	0	0	0	0	0	0
6986	0	**1**	0	0	0	**1**	0	0	0	**1**	0	0
6992	0	0	0	0	0	0	0	0	0	0	0	0

4.4.2.3 Malt Ex

The last Table 46 shows the data for the starvation experiment for the Malt Ex media. With this experiment, five out of seven positive strains (compare with Table 11) could be screened.

Beginning with strain 5492, the bioactivity, after two weeks of cultivation, towards *S. cerevisiae* still remain, but was lost, when the strain was cultivated longer.

As explained in the previous points, this could result due to the degradation of the bioactive metabolites during the growth of the fungus.

The bioactivity versus *E. coli* and *B. velezensis*, after three weeks of cultivation, is for strain 5626 unchanged, but will be lost, when the strain is cultivated for six weeks.

It looks differently, when strain 5628 is cultivated longer than two weeks, because than it will lose its bioactivity against *B. velezensis*.

For strain 5646, no bioactivity can be tested against the four testing strains after two weeks of cultivation.

The bioactivity versus *B. velezensis*, after three weeks of cultivation, is for strain 5712 and 5773 unchanged.

For all tested strains, their ability to produce antimicrobial compounds towards the model organisms was lost after cultivation of six weeks.

Table 46: Summary of results for several strains, grown on Malt Ex and tested after certain timepoints against *E.coli (Ec), B. velezensis (Bv), S. cerevisiae (Sc), F. oxysporum (Fo)*

	14 days				*21 days*				*42 days*			
Strain	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***	***Ec***	***Bv***	***Sc***	***Fo***
5492	0	0	**1**	0	0	0	0	0	0	0	0	0
5626	**1**	**1**	0	0	**1**	**1**	0	0	0	0	0	0
5628	0	**1**	0	0	0	0	0	0	0	0	0	0
5646	0	0	0	0	0	0	0	0	0	0	0	0
5712	0	**1**	0	0	0	**1**	0	0	0	0	0	0
5773	0	**1**	0	0	0	**1**	0	0	0	0	0	0

4.4.3 Synergy of extracts

In the next few tables the results for the synergy of extracts are summarized, where each table is ordered with the strains, that were synergized, in the top line against the strains in the left column. The results for each cell are presented with four number, e.g. 0/1/0/1, which resembles for *E .coli/ B. velezensisv/S.s cerevisiae/F. oxysporum*. The number 1 stands for a positive outcome, which result due to the inhibition of the model organism and the appearance of a halo, otherwise stands the number 0 for no reaction. For this experiment, 5 µL of each strain, which was synergized together was pipetted on blank filter disks.

4.4.3.1 Rice

In Table 47 the result for the synergy experiment of the strain extracts cultivated on rice are summarized. For strain:

5626, 5807 -> Shows towards all used strains an activity to inhibit the growth of one of the used model organisms. All strains show an activity towards *B. velezensis* and E. coli, except for strain 5801and 5490. For strain 5801 and 5831, additionally, an antimicrobial activity towards *S. cerevisiae* could be tested. 5626 against itself shows inhibitory activity versus *E. coli* and *B. velezensis*, so the tested activity wasn´t changed after the synergy experiment, though by riffle the extract of this strain with 5831 and 5801, an activity towards *S. cerevisiae* could be tested. So, when the extracts of strain 5626 and 5831 are mixed together, the growth of three model organisms can be inhibit.

5646, 5828 -> After mixing this strain extract with all other strain extracts, an activity to inhibit the growth towards *B. velezensis* could be seen. Against itself an activity towards *B. velezensis* can be tested, but the mixture with strain extract 5626 and 5807, also an activity versus *E. coli* can be seen. Strain extract 5831 mixed with extract of 5646 an antimicrobial activity to inhibit the growth of *S. cerevisiae* can be achieved.

5494 -> The synergy experiment for this strain, results in an activity to inhibit the growth of *B. velezensis*, after confrontation with all other strains. For strain 5626 and 5807, additionally, an antimicrobial activity towards *E. coli* can be tested. The mixture of this extract with the extracts of 5801 and 5831 result in an inhibitory effect versus *S. cerevisiae*.

5495 -> The results for this strain, looks the same as for strain 5494. The difference here is, that confrontation versus strain 5801 results in an antimicrobial activity only towards *S. cerevisiae* and not versus *B. velezensis*.

5629 -> Shows also quite the same results as written for the two strains before. Strain 5629 shows, after confrontation with strain 5491 additionally, an inhibitory effect versus *S. cerevisiae*.

5767 -> Shows the same results as written for strain 5494.

5801 -> With this strain, an activity to inhibit the growth of model organism *B. velezensis* and *S. cerevisiae* can be tested, when its confronted towards all strains, except of 5646, 6986, 5712 6992, 5491 and 5828. Towards this strains, only an antimicrobial activity versus *B. velezensis* can be seen.
5491 -> This strain shows no inhibitory activity towards the four model organisms. When the extracts are mixed with the other strains, except for 6986, 5712, 6992, 5491, an antimicrobial activity versus *B. velezensis* can be tested. Additionally, to that, an activity towards *E. coli* can be checked, when its confronted with strain extract 5626 and 5807 and for strain extract 5801 and 5831 an inhibitory activity against *S. cerevisiae* .

5490 -> Shows antimicrobial activity, after mixing with all other extracts, towards *B. velezensis*. Mixing with strain extract of 5801 results also in an inhibitory effect versus *S. cerevisiae*.

6986, 5712, 6992 -> No reaction can be seen, when this extract is tested towards the four model organisms. But when this extract is tested in combination with elven strain extracts, results in a reaction towards *B. velezensis*. Also, an inhibitory reaction can be tested versus *E. coli*, when strain extracts of 5626 and 5807 is used.

5831 -> With this extract, a mixture with all other strains result in an antimicrobial activity towards one of the four model organisms. Versus all strains, an inhibitory effect against *B. velezensis* and *S. cerevisiae*, except towards 5490, can be tested. Also, two antimicrobial activities versus *E. coli* can be seen, when mixture of this extracts with strain extract of 5807 and 5626 are taken.

5492 -> Shows no reaction, when its tested alone towards the model organisms. Mixture with the other strains, except for 5491, 5712, 6986 and 6992, result in an antimicrobial activity at least towards one testing organism.

Table 47: Summarized results for the synergy of extracts, gained after lyophilization of strains, cultivated on Rice and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	*5626*	*5646*	*5494*	*5495*	*5629*	*5767*	*5801*	*5491*	*5490*	*6986*	*5712*	*6992*	*5831*	*5492*	*5807*	*5828*
5626	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	0/1/1/0	1/1/0/0	0/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/1/0	1/1/0/0	1/1/0/0	1/1/0/0
5646	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
5494	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
5495	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/0/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
5629	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
5767	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
5801	0/1/1/0	0/1/0/0	0/1/1/0	0/0/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/1/0	0/1/0/0
5491	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/1/1/0	0/0/0/0	1/1/0/0	0/1/0/0
5490	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0
6986	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/1/1/0	0/0/0/0	1/1/0/0	0/1/0/0
5712	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/1/1/0	0/0/0/0	1/1/0/0	0/1/0/0
6992	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/1/1/0	0/0/0/0	1/1/0/0	0/1/0/0
5831	1/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/0/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	0/1/1/0	1/1/1/0	0/1/1/0
5492	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	0/1/0/0	0/0/0/0	0/1/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/1/1/0	0/0/0/0	1/1/0/0	0/1/0/0
5807	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	0/1/1/0	1/1/0/0	0/1/0/0	1/1/0/0	1/1/0/0	1/1/0/0	1/1/1/0	1/1/0/0	1/1/0/0	1/1/0/0
5828	1/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/0/0	0/1/1/0	0/1/0/0	1/1/0/0	0/1/0/0
Total Ec/Bv/Sc/Fo	14/16/2/0	2/16/1/0	2/16/2/0	2/15/2/0	2/16/3/0	2/16/2/0	0/15/10/0	2/11/2/0	0/16/1/0	2/11/1/0	2/11/1/0	2/11/1/0	2/16/15/0	2/11/1/0	14/16/2/0	2/16/1/0

4.4.3.2 Dox

In Table 48 the results for the synergy effect of different extracts gained after cultivation on Dox. For strain:

5626 -> Shows, when its tested against the four model organisms, antimicrobial activity versus *B. velezensis*. Mixtures with other extracts gained out of Dox medium, results in no reaction versus the testing organisms.

Table 48: Summarized results for the synergy of extracts, gained after lyophilization of strains, cultivated on Dox and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	*5490*	*5626*	*5808*	*5712*	*6992*	*5491*	*5773*	*5495*	*5628*
5490	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5626	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5808	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5712	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
6992	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5491	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5773	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5495	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5628	0/0/0/0	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
Total Ec/Bv/Sc/Fo	**0/1/0/0**	**0/9/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**

4.4.3.3 Malt Ex

Table 49 shows the results of extracts gained out of Malt ex medium. Strain extracts 6986, 5646, 5712, 5628, 5773 and 5492 shows no antimicrobial activity towards the four model organisms. For strain:

6037 -> Shows towards all other strain extracts an inhibitory reaction versus *B. velezensis*. With strain 5626 it shows additionally an antimicrobial activity against *E. coli*.

5626 -> As written for strain 6037, this strain extract shows antimicrobial activity towards *E. coli* and *B. velezensis*, when its mixed with all other strains.

Table 49: Summarized results for the synergy of extracts, gained after lyophilization of strains, cultivated on Malt Ex and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	*6037*	*5626*	*6986*	*5646*	*5712*	*5628*	*5773*	*5492*
6037	**0/1/0/0**	**1/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**
5626	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**
6986	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5646	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5712	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5628	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5773	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5492	**0/1/0/0**	**1/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
Total Ec/Bv/Sc/Fo	**1/8/0/0**	**8/8/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**

4.4.3.4 PDA

All results for the synergy experiment with extracts gained from PDA medium can be seen in Table 50. For strain:

5828 -> With this extract, no antimicrobial activity can be seen against the four model organisms, but when its mixed with strain extract of 5626 and 5807 an activity versus *B. velezensis* and *E. coli*.

5789, 5779, 5628, 5773, 5767 -> shows the same reactions as written for strain 5828.

5767, 6183, 5830, 5806, 5835 -> shows the same reactions as written for strain 5828 plus an inhibitory effect towards *S. cerevisiae* , when extracts were mixed with strain 5490.

5626 -> With this strain extract, mixtures with all other strains show an antimicrobial activity towards *B. velezensis* and *E. coli*. Additionally, to that, with strain 5807 and 5490, an inhibitory effect towards *S. cerevisiae* can be tested.

5807 -> Shows inhibitory effect towards *B. velezensis*, when the extracts are mixed with all strain extracts. Also, an antimicrobial activity versus *S. cerevisiae* can be seen, with mixtures of 5807 and 5626/5635/5490. Only one reaction to inhibit the growth of *E. coli* can be measured, when strain extract 5626 is used in combination with 5807.

5490 -> With this strain extracts, a reaction can be seen to inhibit the growth of *S. cerevisiae* , when its combined with most of the used extracts. Additionally, to that, an antimicrobial activity towards *E. coli* and *B. velezensis* can be tested, when this extract is mixed with strain 5626 and 5807.

Table 50: Summarized results for the synergy of extracts, gained after lyophilization of strains, cultivated on PDA and tested against *E.coli* (Ec), *B. velezensis* (Bv), *S. cerevisiae* (Sc), *F. oxysporum* (Fo)

Strain	*5828*	*5789*	*5779*	*5628*	*5773*	*5767*	*6183*	*5626*	*5807*	*5830*	*5806*	*5635*	*5490*
5828	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5789	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5779	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5628	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5773	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0
5767	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**
6183	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**
5626	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/1/0**	**1/1/0/0**	**1/1/0/0**	**1/1/0/0**	**1/1/1/0**
5807	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**0/1/0/0**	**1/1/1/0**	**0/1/1/0**	**0/1/1/0**	**0/1/1/0**	**0/1/1/0**	**0/1/1/0**
5830	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**
5806	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/0/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**
5635	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**1/1/0/0**	**0/1/1/0**	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**
5490	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	**0/0/1/0**	**0/0/1/0**	**1/1/1/0**	**0/1/1/0**	**0/0/1/0**	**0/0/1/0**	**0/0/1/0**	**0/0/1/0**
Total Ec/Bv/Sc/Fo	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/0/0**	**1/2/1/0**	**1/2/1/0**	**13/13/1/0**	**1/13/4/0**	**1/2/2/0**	**1/2/2/0**	**1/2/2/0**	**1/2/8/0**

5 Concluding Remarks

Forty isolated fungal strains from the high canopy of the low land rainforest of Borneo were tested on ten different nutritional media (PDA, Dox, YES, YESD, Malt Ex, YPSS, YM, Rice, Oat Flour, PYG) in this thesis. Fifteen of them, showed an antimicrobial activity against at least one of the four used model organisms (*E. coli*, *B. velezensis*, *S. cerevisiae*, *F. oxysporum*), after cultivation on one of the media. The antimicrobial activity of the fungal ASMs could be covered by five out of the ten selected media, which includes PDA and Malt Ex (as best anti-fungal media) and Dox, YESD, Rice media. The Dox medium was the best in production of antibacterial agents against both *B. velezensis* and *E. coli* strains and more than 50% (8 of 15 positive strains) of all the ASM producers were shown inhibitory activity against them. *P. expansum* (TUCIM 5626, Guochun *et al.,* 2004), *Ovicillium* sp. (TUCIM 5628), *Xylaria* sp. (TUCIM 5712, Ramesh *et al.,* 2012) and *Arthrinium rasikravindrii* (TUCIM 5773, Pansanit and Pripdeevech 2018) showed over all used nutritional conditions the best antimicrobial activity against the four model organisms.

The extraction of the antimicrobial metabolites out of the cultivated strains could be done, with a mixture of methanol/dichloromethane (1:2), in which the methanol extracted the polar and the dichloromethane the non-polar compounds. The antimicrobial activity tested on the rice medium, with plug test compared to the extraction of the antimicrobial compounds revealed, that out of forty tested strains, five showed an antimicrobial activity against gram + and gram – bacteria, when they were tested with the plug test and ten, when they were tested as extracts. Additionally, with the extracts, an inhibitory effect against *S. cerevisiae* could be tested with two of the ten antimicrobial metabolite producers. The difference in results between the plug test and the extraction could have several reasons, one of the main would be the concentration of the metabolites and the cultivation time. The extraction concentrated the metabolites to a final volume of a few hundred µL, compared to the plug test, where the plugs taken randomly over the plate. The same result could be observed, when the extraction was done with the PDA medium, but showed less success with the media Malt Ex and Dox.

With the Minimum Inhibitory Concentration (MIC), tested in 96 deep well microplates, the antimicrobial activity could be numbered. *P. expansum* (TUCIM 5626) showed in Malt Ex a MIC range of 128 µg/mL and in PDA 64 µg/mL, against *E. coli*. Additionally, a MIC range of 128 µg/mL in Malt Ex, 32 µg/mL in PDA and 1024 µg/mL in Dox, against *B. velezensis* could be measured.

Arthrinium rasikravindrii (TUCIM 5773) showed a MIC range of 128 µg/mL in Malt Ex and 32 µg/mL in PDA, against *B. velezensis*.

Xylaria sp. (TUCIM 5712) was also tested and showed a MIC range 256 µg/mL in Malt Ex and 64 µg/mL in Rice, against *B. velezensis.* For *Ovicillium* sp. (TUCIM 5628) only in PDA a MIC range of 128 µg/mL, against *B. velezensis*, could be tested.

As mentioned, the cultivation time, as a stress factor, plays a key role in the production of antimicrobial metabolites and therefore, was tested over six weeks. The antimicrobial compounds could be tested, after 14 days of cultivation on PDA, except for *Arthrinium rasikravindri* (TUCIM 5773), which showed activity also, after 21 days. For Malt Ex and Dox, mainly all tested strains showed an activity, after 21 days of cultivation, *Paracremonium* sp. (TUCIM 5491) and *Chaetosphaeria* sp. (TUCIM 6986), even after 42 days, when they were cultivated on Dox.

The co- culture experiment showed, that an adaption of antimicrobial metabolite production is possible and could be achieved with several strains, like *P. expansum* (TUCIM 5626) with *Paracremonium* sp. (TUCIM 5491) in Dox medium and *Xylaria* sp. (TUCIM 5712) with *Arthrinium rasikravindrii* (TUCIM 5773) in YESD medium. A change in the size of inhibitory cone with extract mixtures in the synergy experiment, couldn´t be tested with neither one isolated fungi strain.

6 Outlook

In this thesis we concerned more about finding an antimicrobial metabolite producer against gram +, gram – bacteria, yeast and filamentous fungi, out of a selected fungi pool from the rainforest of Borneo, the habitat of exploding ants, than to identify them. Therefore, for the next steps, the identification of the antimicrobial compounds, produced by the screened fungi, have to be determined. Hajime *et al.,* (1994) described a way to purify and identify the produced antimicrobial metabolites. They used in their study, a chromatographic system, with a silica gel column and a mixed solvent of hexane-EtOAc (7:3) to EtOAc, to obtain different fractions. By using another chromatographic system, those fractions were separated again and purified with an attached preparative HPLC. The purified metabolites were identified with an ^{1}H-NMR method. Between Hajime *et al.,* paper and now day 24 years gone by and the technology changed a lot, why new papers are published with easier methods to analyse and identify the antimicrobial metabolite. For example, Maree *et al (2014).* published a method to analyse the compounds with an GC system, which was coupled with an MS instrument, to identify the analysed metabolites.

Another step to characterise the produced antimicrobial compounds in an efficient way, should be, by testing them against a broad spectrum of microorganisms, that includes non-pathogens and pathogens (Falaise *et al.,* 2016). Additionally, the produced antimicrobial metabolites have to be tested, if they affect the human organism and when so, how the metabolism will be changed.

Are those barriers taken, a genome analyses of the antimicrobial metabolite producers should be done, to get an idea, which genes or gene clusters are involved in the synthesis of those secondary metabolites. One method to find those clusters would be the gene knockout, where stepwise active genes are silenced and screened for the production of the secondary metabolites afterwards. With this knowledge, those genes/gene clusters could be transferred into an organism, that would produce those secondary metabolites in a higher yield.

7 Curriculum Vitae

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Education

November 2016 – December 2018
Master´s degree – Biotechnology and Bioanalytic at the Technical University of Vienna
Master thesis: „Antimicrobial activity of the rare and novel fungi, isolated from the high canopy of Borneo rainforest"

October 2012 – November 2016
Bachelor´s degree – Technical Chemistry at the Technical University of Vienna
Bachelor thesis: „Methodenentwicklung für enzymatischen Verdau auf Gewebeproben, mittels Trypsin/Lys-C - Probenvorbereitung für massenspektrometrisches Imaging"

September 2007 – July 2012
High School - HBLVA Rosensteingasse

Work experience

April 2016 – September 2016
Internship at OMV- department of MRDT-N New Technology

August 2011
Internship at Federal environmental agency of Vienna

Juli 2009 / Juli 2010
Internship at Rembrandtin Lack GmbH Nfg. KG

Qualifications

Language
German (native), Englisch (fluent)

IT knowledge
ECDL Base and EBCL Step A and B

Interests
Friends and family, dancing, baseball,

8 References

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9 Appendix

9.1 Minimum Inhibitory Concentration

9.1.1 *Arthrinium* sp. (TUCIM 5807)

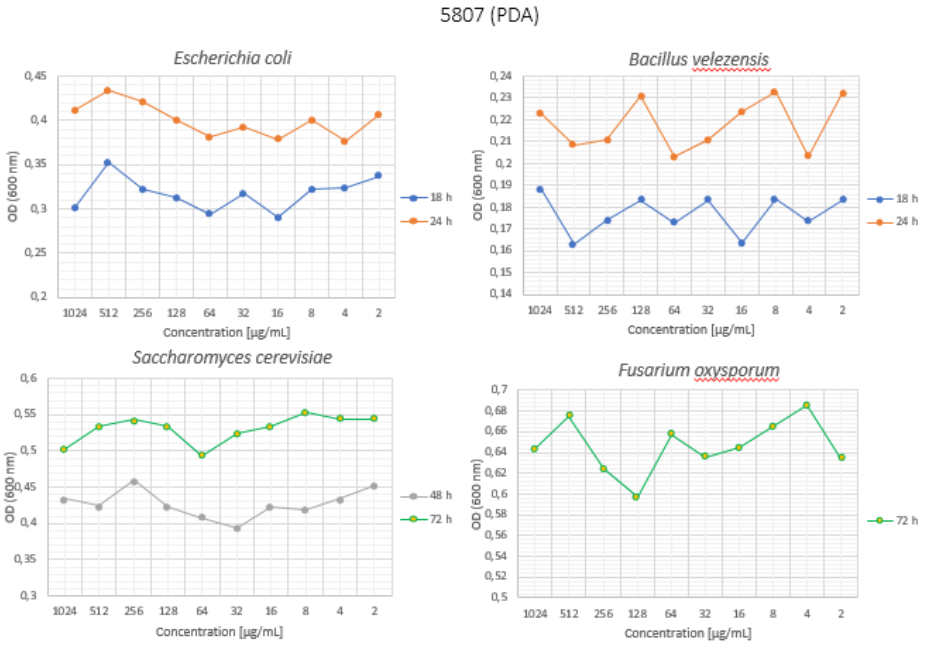

Figure 24: MIC experiment for the strain extract 5807, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.2 *Trichothecium* sp. (TUCIM 5490)

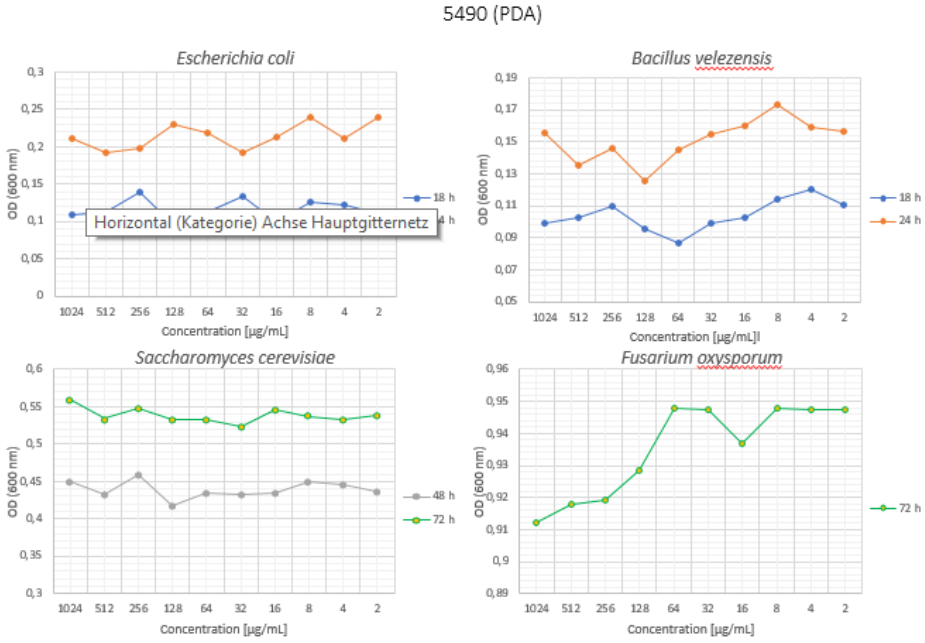

Figure 25: MIC experiment for the strain extract 5490, cultivated on PDA and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.3 *Ovicillium* sp. (TUCIM 5628)

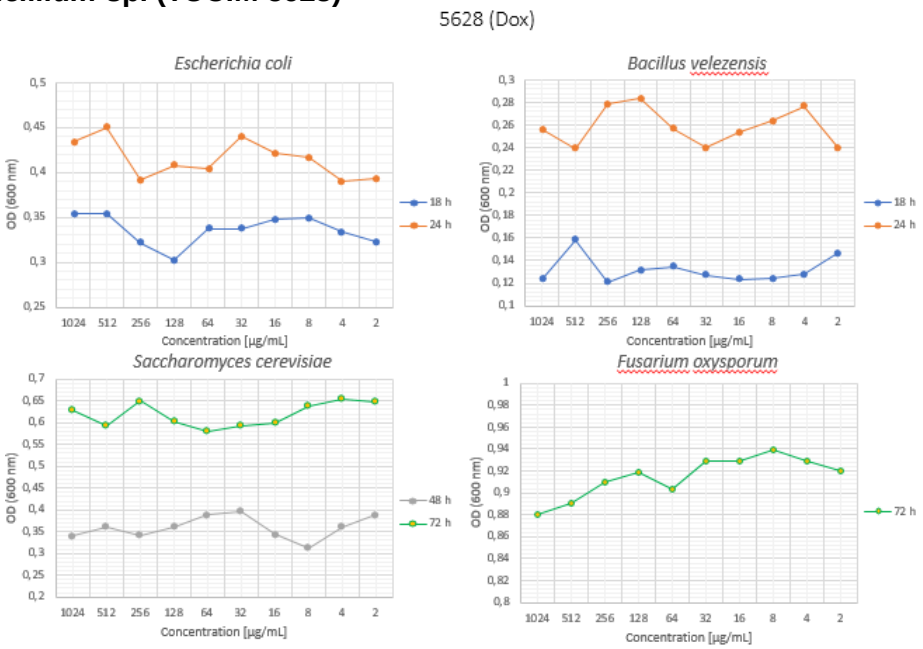

Figure 26: MIC experiment for the strain extract 5628, cultivated on Dox and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

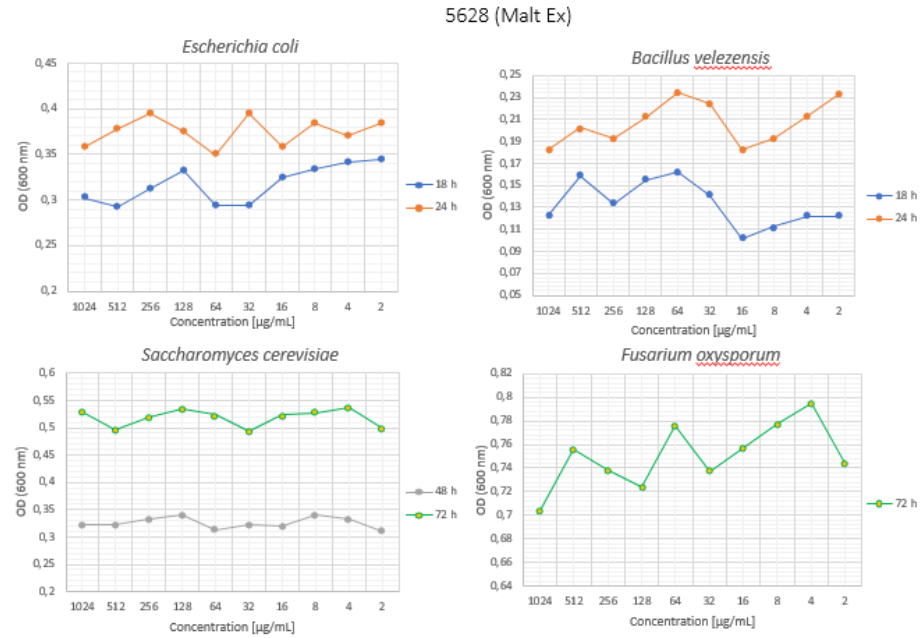

Figure 27: MIC experiment for the strain extract 5628, cultivated on Malt Ex and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.4 *Xylaria* sp. (TUCIM 5712)

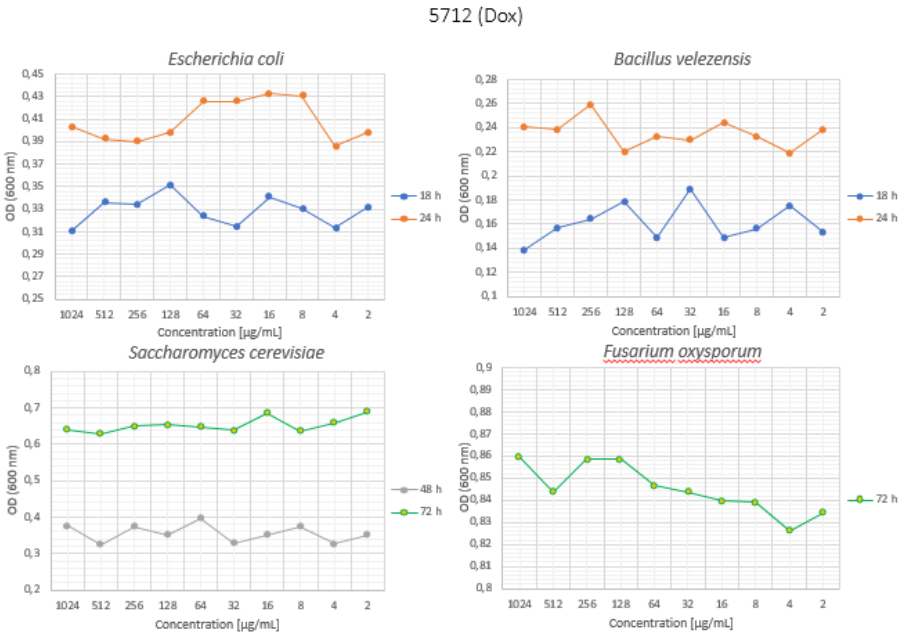

Figure 28: MIC experiment for the strain extract 5712, cultivated on Dox and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.5 *Arthrinium rasikravindrii* (TUCIM 5773)

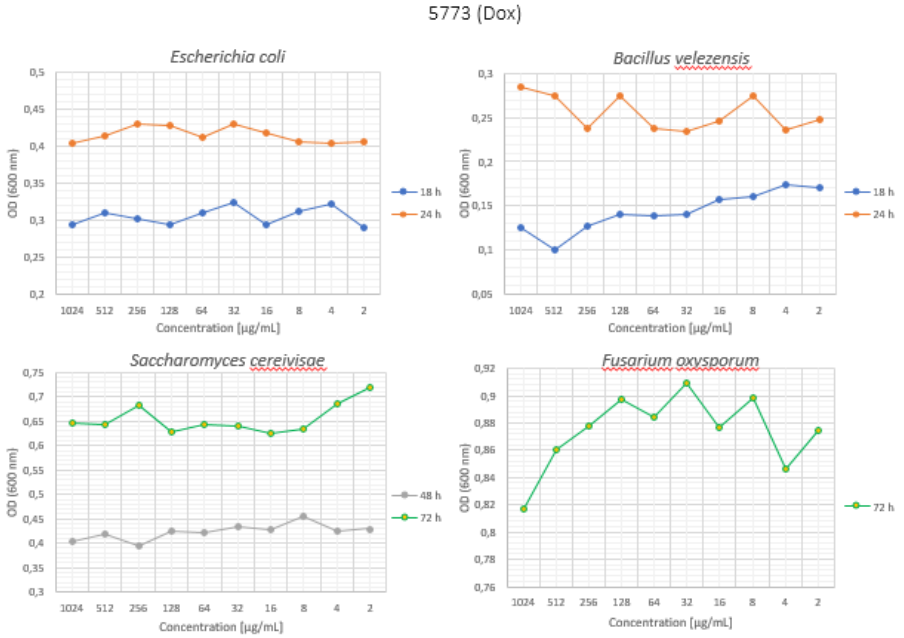

Figure 29: MIC experiment for the strain extract 5773, cultivated on Dox and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.6 *P. expansum* (TUCIM 5626)

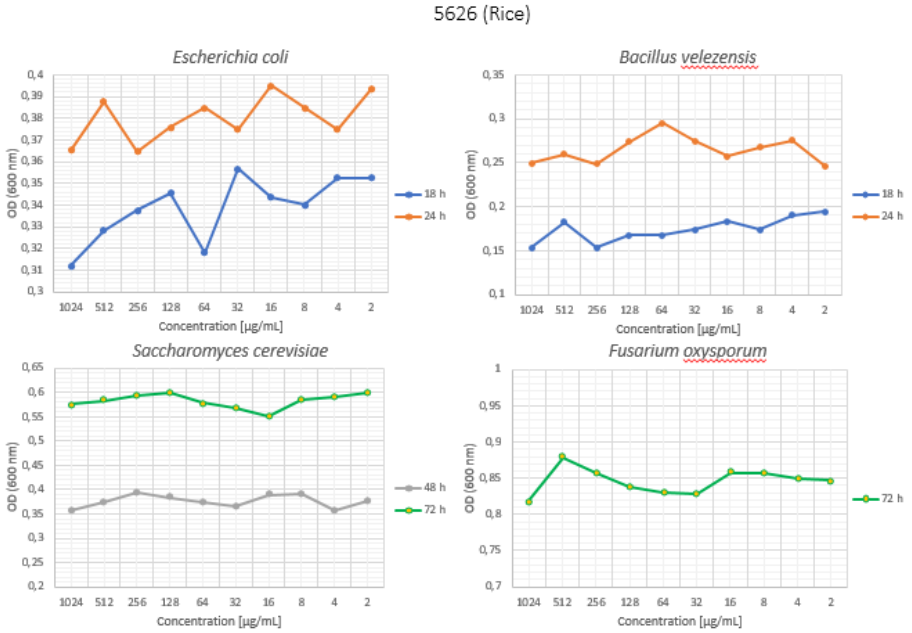

Figure 30: MIC experiment for the strain extract 5626, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.7 cf. *Leotiomycetes* sp. (TUCIM 5828)

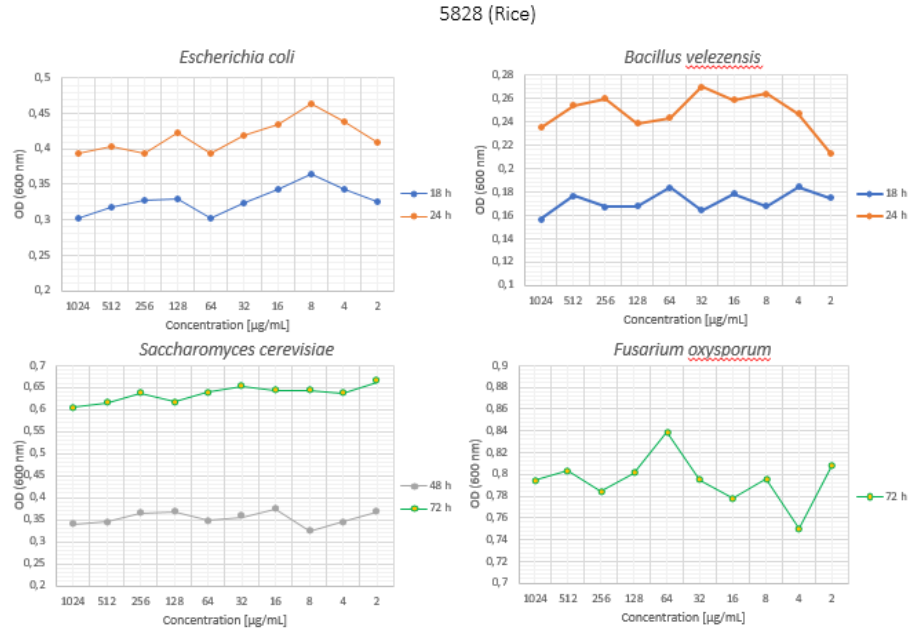

Figure 31: MIC experiment for the strain extract 5828, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*

9.1.8 *Pestalotiopsis* sp. (TUCIM 5495)

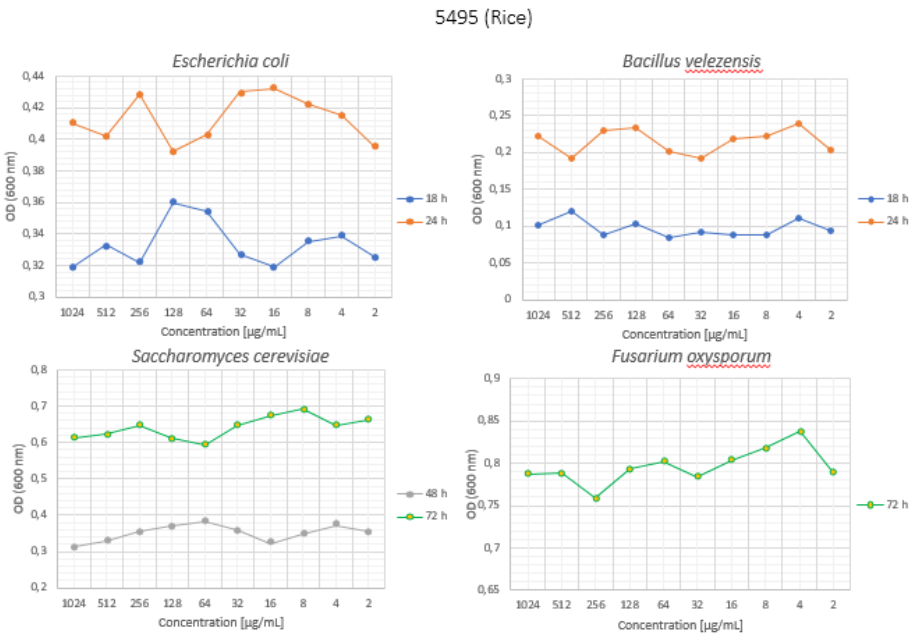

Figure 32: MIC experiment for the strain extract 5495, cultivated on Rice and tested against *E. coli*, *B. velezensis*, *S. cerevisiae* and *F. oxysporum*