Bachelorarbeit

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Intraspecific variations of *Cryptococcus victoriae* in response to changes in sugar concentration

Intraspezifische Variationen von *Cryptococcus victoriae* in Abhängigkeit von Veränderungen der Zuckerkonzentration

von

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1. Introduction

The systematics of the genus *Cryptococcus* are still unclear, its members are grouped into an artificial assemblage that occurs in the clades Cystofilobasidiales, Filobasidiales, Tremellales and Trichosporonales (Fell et al., 2000). Species of this genus can be found in most habitats on earth, including sites with extreme conditions such as glaciers, polar deserts (Vishniac, 1985, Brizzo et al., 2007, de García et al., 2010) and hot deserts (Baum et al., 1965, Sneller et al., 1974).

Cells of *Cryptococcus* are described in "The Yeasts: A taxonomic study" as generally being spheroidal, ovoid, ellipsoidal or elongate in shape, with a polysaccharide capsule present in most species. Reproduction occurs asexually through multilateral or polar enteroblastic budding (see Figure 1). Sexual reproduction has not yet been described, but some species represent the anamorphic states of teleomorphic genera in the clades Cystofilobasidiales, Filobasidiales and Tremellales. It may develop pseudohyphae or true hyphae, the latter having septa with dolipores and potentially parenthosomes. They do not, however, form arthroconidiae or ballistoconidiae. Colors on solid media are white, cream or tan, but may develop red, orange, yellow or dark brown pigmentation. Their texture ranges from mucoid to butyrous (Fell et al. 2011). All species of the genus described by Benham as well as Kreger-van Rij later on are able to assimilate Glucose and Galactose, and all of them can assimilate at least several other types of sugar as well, hinting at a genus of generalist yeasts rather than specialists (Benham, 1955 & Kreger-van Rij, 1964).

Cryptococcus is highly important to medical science because some of its species are of medical relevance as human pathogens, especially *Cryptococcus neoformans* (Vuillemin, 1901) and *Cryptococcus gattii* (Kwon-Chung et al., 1982), agents of Cryptococcosis, a cutaneous, pulmonary or neurological opportunistic infection (Kauffman et al., 2007 and Baddley et al., 2008). Cryptococcus has also been mentioned in regards to its use in biotechnology, including its potential as a 'workhorse' to produce novel enzymes and biomolecules, e.g. agents for xenobiotic breakdown or new pharmaceutical chemicals (Shivaji & Prasad, 2009).

Cryptococcus victoriae, the species this thesis primarily deals with, was first described based on samples collected from Botany Bay (Southern Victoria Island, Antarctica) in 1999. Its GC-content was stated as about 50.3%, and phylogenetic analysis of the 5.8S rRNA suggested the

new strain a species of the genus *Cryptococcus*, therefore named *Cryptococcus victoriae* (Montes et al., 1999). The description further contains a cell size of 3 x 2 µm, cream-colored colonies of slimy appearance, no apparent mycelia or pseudomycelia and no apparent sexual reproduction. Assimilated compounds include, among other things, several sugars, sugar alcohols and types of starch, depicted on Table 1. Growth temperature was stated as between 5°C and 20°C, with an optimum at 15°C. Three additional novel strains of *Cryptococcus victoriae*, collected in 1997 from Vestvold Hills (Davis Base, Antarctica), have been described as having a cell size of 3-5 x 2-3 µm, with large cells of up to 10 x 4 µm having been formed occasionally (Thomas-Hall et al., 2002). The cells exhibited polar budding (see Fig. 1). Habitus of colonies was described as convex to umbonate, irregular and smooth, with colors ranging from pink to apricot or cream and of butyrous consistence. Later samplings show that the species can also be found in Patagonia, Argentina and Svalbard, Norway (de Garcia et al., 2012), as well as Germany (Yurkov et al., 2010 & Prior, 2012) and Tenerife (Mittelbach, 2012).

Table 1: Assimilation tests conducted on four different strains of *C. victoriae* (Thomas-Hall et al., 2002)

Growth test	**1**	**2**	**3**	**4**
$_{L}$-Sorbose	-	w	-	-
Cellobiose	+	+	+	w
Lactose	+	+	+	-
Soluble starch	w	s/w	-	-
$_{L}$-Arabinose	+	w	+	+
$_{L}$-Rhamnose	+	w	+	+
$_{D}$-Glucosamine	w	w	+	w
Galactitol	+	+	w	w
Methyl α-$_{D}$-glucoside	+	+	+	w
Succinate	+	w	w	+
Citrate	+	w	w	+
Inositol	+	w	w	-
Vitamin-free medium	+	w	+	w
$_{D}$-Glucuronate	+	w	+	w
10% NaCl, 5% Glucose	w	w	+	+
50% (w/w) Glucose / Yeast extract agar	-	-	w/-	w

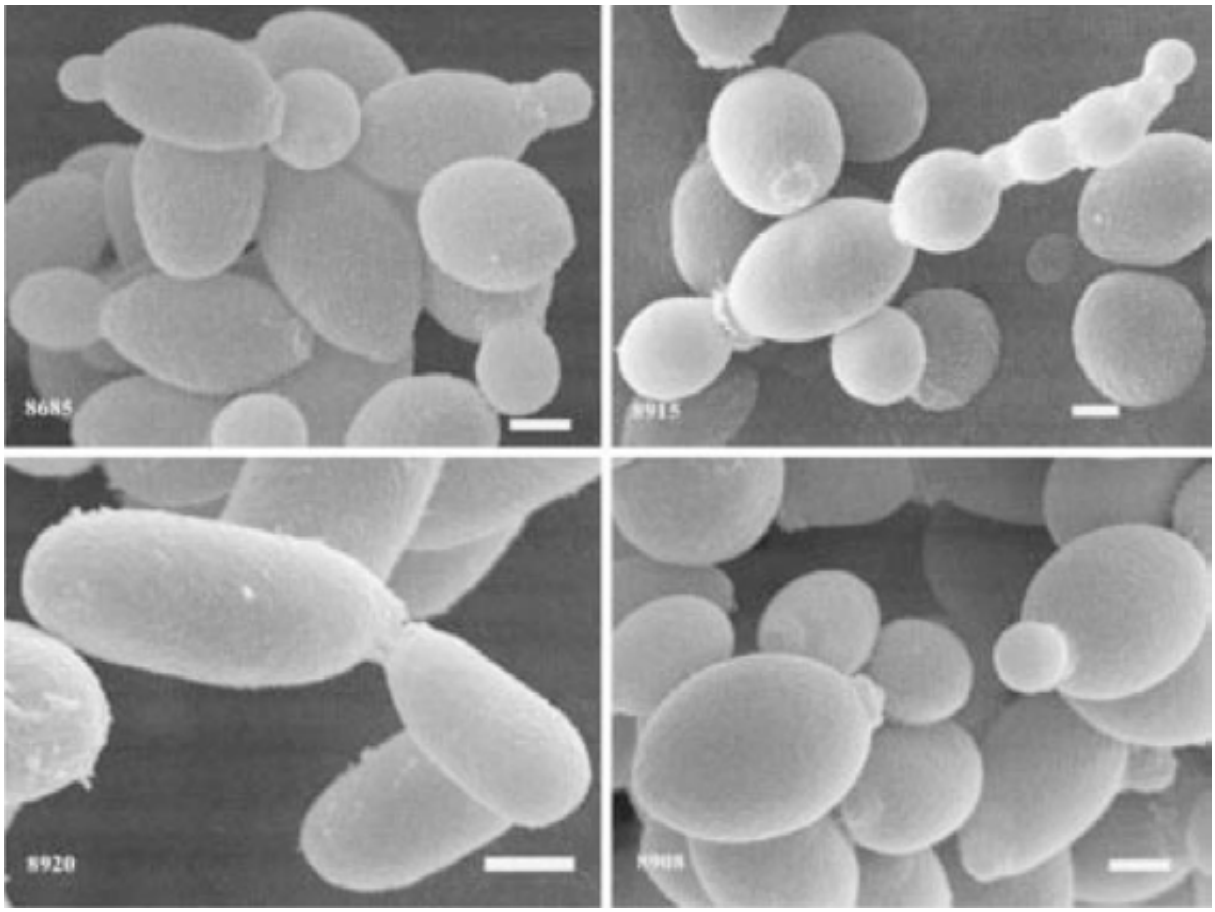

Figure 1: Electron microscopic scanning of various *Cryptococcus victoriae* strains by Thomas-Hall et al., showing budding cells and resulting bud scars. Image cropped. Bars are 1 µm.

Cryptococcus victoriae, a generalist in terms of sustenance and environment, has been found in many habitats, the nectar of flowering plants among them. In this merotope, it is likely subject to competition with highly specialized microorganisms, several species of the genus *Metschnikowia* among them (Kabir et al., 2011). *Metschnikowia* is, unlike *Cryptococcus*, a genus of ascomycetous yeasts that reproduces via multilateral budding or up to two distinct, needle-shaped ascospores per ascus during their sexual state (Miller, Barker and Pitt, 1967; Mendonça-Hagler et al., 1993). Cells are usually spherical to ellipsoid, but cylindroids, lunate or even distinct 'plane-shaped' cells can be found (Pitt & Miller, 1968; Lachance et al., 2011). *Metschnikowia* species grow well on various agar media containing sugar, but less so on other media (Lachance et al., 2011). This genus was first described by Ilja I. Metschnikow in 1884 on *Daphnia magna*, and up to this date 42 Metschnikowia species and 26 Candida species of aquatic as well as terrestrial origin have been described (Lachance et al., 2011; de Vega et al., 2012; Kaewwichian et al., 2012). Some of them, such as *M. gruessii* or *M. reukaufii*, are found regularly in the nectar of flowering plants, with increasing distribution and density as

the year progresses, and have been proven to establish a clear predominance in this habitat during tests in south-eastern Spain (Herrera et al., 2009 and 2012; Pozo et al, 2011).

Nectar is considered as a harsh habitat, providing high sugar concentrations that yield massive osmotic stress. Additionally, floral microorganisms have to deal with a large variety of plant induced antimicrobial compounds as well as temporal and spatial fragmentation due to the nature of nectaries. Nectar is secreted from flowers to attract pollinating animals, mainly insects and birds. It is mainly composed of sucrose, glucose and fructose, with mixture ratio from plant species to species (Nicolson et al., 2007), and may contain various other minor components like pollinator-attracting volatiles or toxins to prevent contamination (Ecroyd et al, 1995).

All strains used in this thesis were collected in spring 2012 on the island of Tenerife during a study focusing on nectariferous yeasts in flowers of several species of the genus *Echium* (Boraginaceae). Floral habitats show different characteristics due to shifts in pollinations syndromes, such as nectar volumes and sugar concentrations. Nectar of *Echium plantagineum* has an average sugar concentration of about 20 to 50% (Corbet and Delfosse, 1984), *E. strictum* of 50 to 70% (30 to 50% post-production) and *E. leucophaeum* of 10 to 30% (Mittelbach, 2010, unpublished). Still, these flowers do not primarily contain the aforementioned species of *Metschnikowia*, but strains of *Cryptococcus victoriae* are found as most abundant, even though high concentrations of sugar usually deter such generalist yeast species. In regard to *Cryptococcus victoriae*, this raises the following four questions:

I. What are the responses of *Cryptococcus victoriae* to various different sugar concentrations?

The basidiomycete *Cryptococcus victoriae* exists ubiquitous in various places, soil, nectar or the phyloplane of plants among them, suggesting that it is no nectar specialist. As such, the question is being raised whether this species shows distinct reaction to changes in the concentration of sugar while occupying such a habitat. If so, different concentrations of its nutrition medium should influence the growth rate of the strains.

II. How much variation exists within a species?

Should *Cryptococcus victoriae* prove itself to be of higher tolerance towards fluctuation of sugar concentration, does the species show intraspecific distinctions? Higher or lower concentrations of sugar could have different impact on the various *C. victoriae* strains, resulting in specific growth rates for each strain.

III. Can a habitat-induced predisposition be discerned?

If the different strains exhibit different growth rates in media of the same sugar concentration, are these intraspecific distinctions attributable to varieties in the habitat's concentration of sugar from which they were sampled?

IV. Do specialists for sugar-based habitats exist among *C. victoriae*?

Is it even possible that *Cryptococcus victoriae* can adapt itself to high-sugar environments in a way that it can be considered a specialist like e.g. *Metschnikowia*?

2. Material and Methods

2.1 Laboratory Equipment

2.1.1 Devices

Axiostar plus light microscope	Carl Zeiss AG, Jena, Germany
AB304-S micro scales	Mettler-Toledo, Greifensee, Swiss
Centrifuge 5424	Eppendorf, Hamburg, Germany
Epoch Microplate Reader	BioTek, Winooski (VT), USA
µQuant Microplate Reader	BioTek, Winooski (VT), USA
Neubauer improved counting chamber	Marienfeld, Lauda-Königshofen, Germany
shaker SM-30	Edmund Bühler GmbH, Hechingen, Germany
Vortex-Genie® 2	Scientific Industries, Bohemia, USA

2.1.2 Software

Gen5 (Ver. 1.04.5)	BioTek, Winooski (VT), USA
Microsoft Office 2007	Microsoft Corporation, Redmond, USA
Photoshop Elements CS6	Adobe Systems Inc., San Jose (Cal), USA
R (Ver. 2.15.2); Package Vegan	R Development Core Team
SPSS (Ver. 21.0)	IBM Corporation, Endicott, USA

2.1.3 Consumption equipment

Microtest Plate 96Well F	Sarstedt, Nümbrecht, Germany
Breathe-Easy® Sealing Membrane	Diversified Biotech, Boston, USA
Parafilm	Pechiney Plastic Packaging, Chicago, USA
Glass Beads, 0.25-0.5 mm	Carl Roth, Karlsruhe, Germany

2.1.4 Chemicals

Agar	AppliChem, Darmstadt, Germany
Ethanol	Sigma-Aldrich, St. Louis, USA
Fructose	AppliChem, Darmstadt, Germany
Glucose	AppliChem, Darmstadt, Germany
Malt Extract	Merck KGaA, Darmstadt, Germany
Potato-Extract-Glucose Agar	Merck KGaA, Darmstadt, Germany
Sucrose	AppliChem, Darmstadt, Germany
Yeast Extract	AppliChem, Darmstadt, Germany
Yeast Nitrogen Base	Fluka (Sigma-Aldrich), St. Louis, USA

YM solid medium:	2% (w/v) agar 1% (w/v) glucose 0.3% (w/v) malt extract 0.5% (w/v) peptone/soyone 0.3% (w/v) yeast extract	Prepared in a wide neck bottle + agitator, with desalted water, autoclaved for ~20 min. at 121°C
YMPD solid medium:	2% (w/v) agar 1% (w/v) glucose 0.3% (w/v) malt extract 0.5% (w/v) peptone/soyone 2% (w/v) potato-extract-gluc. agar 0.3% (w/v) yeast extract	Prepared in a wide neck bottle + agitator, with desalted water, autoclaved for ~20 min. at 121°C
YNB medium:	0.67% (w/v) yeast nitrogen base	Prepared in a wide neck bottle + agitator, with desalted water, autoclaved for ~20 min. at 121°C
YNB + 3S medium:	0.67% (w/v) yeast nitrogen base 1% (w/v) glucose 1% (w/v) sucrose 1% (w/v) fructose	Prepared in a wide neck bottle + agitator, with desalted water, autoclaved for ~20 min. at 121°C

2.2 Methods

10 of the pure cultures used had been collected from plants of the genus *Echium* on Tenerife by Moritz Mittelbach in 2012, while three others were acquired from different sources: TSN 486 and TSN 529 come from soil samples collected by Thorsten Wehde, and 2A-28 epiphytic from Bochum, Germany (course material, collected from *Taraxacum officinale*). All of these had been stored in glycerin-filled 2ml micro test tubes prior to experimentation at -80°C.

To test potential growth trajectories, cell cultures were to be incubated in solutions of varying sugar concentration, namely 0% (pure YNB medium), 10%, 20%, 30%, 40% and 50% sugar. This solution, a combination of glucose, fructose and sucrose with equal percentage composition dissolved in liquid YNB-medium, is hereinafter referred to as GFS-medium. Testing took place using 96-well-micro-testplate at room temperature. As each well was to be filled with 20 µl cell culture and 80 µl GFS-medium, the latter's concentration was set slightly higher to ensure the desired final concentration.

Yeast samples taken out of deep cooling were spread on YM-solid media in petri dishes, sealed with parafilm, and incubated at room temperature for at least 3 days, but no more than two weeks. Cells removed from these plates via sterile inoculation loops were placed in micro reaction tubes filled with 1 ml YM +3S medium, mixed with a vortex and transferred into a test tube filled with another 1 ml of YM +3S medium. This procedure was repeated with a second test tube for redundancy. Both tubes were incubated on a shaker at 225 movements per minute for 24 hours.

The next day, two 1.5 ml micro test tubes were filled with 800 ml each from one and the same test tubes inoculated the day prior. Each micro test tube was subsequently centrifuged at 2000 rpm for 5 minutes. The supernatant liquid was taken off and the remaining mass of cells washed with 800 ml sterile-filtered tap water. The whole procedure was repeated, centrifuging with the same rotation speed and duration, taking off the supernatant and washing with sterile tap water. A 1:1000 solution of the resulting liquid was then used to determine the cell count using a Neubauer improved counting chamber. The resulting cell number was diluted to achieve the desired number of 400 cells per micro liter. Afterwards, one 96-well-plate was prepared for each run using the following pattern:

Table 2: Filling pattern of 96-well-plates

BK	BK	BK	BK	BK	BK	R8 0%	R8 10%	R8 20%	R8 30%	R8 40%	R8 50%
R1 0%	R1 10%	R1 20%	R1 30%	R1 40%	R1 50%	R9 0%	R9 10%	R9 20%	R9 30%	R9 40%	R9 50%
R2 0%	R2 10%	R2 20%	R2 30%	R2 40%	R2 50%	R10 0%	R10 10%	R10 20%	R10 30%	R10 40%	R10 50%
R3 0%	R3 10%	R3 20%	R3 30%	R3 40%	R3 50%	R11 0%	R11 10%	R11 20%	R11 30%	R11 40%	R11 50%
R4 0%	R4 10%	R4 20%	R4 30%	R4 40%	R4 50%	R12 0%	R12 10%	R12 20%	R12 30%	R12 40%	R12 50%
R5 0%	R5 10%	R5 20%	R5 30%	R5 40%	R5 50%	R13 0%	R13 10%	R13 20%	R13 30%	R13 40%	R13 50%
R6 0%	R6 10%	R6 20%	R6 30%	R6 40%	R6 50%	R14 0%	R14 10%	R14 20%	R14 30%	R14 40%	R14 50%
R7 0%	R7 10%	R7 20%	R7 30%	R7 40%	R7 50%	R15 0%	R15 10%	R15 20%	R15 30%	R15 40%	R15 50%

Each row of six wells equals one repeat, with the corresponding concentration of GFS stated below the row label (R^n). Every well except the blanks (labeled BK) was filled with 80 ml GFS medium and 20 ml of the solution containing 400 cells per micro liter. The blanks were filled with 80 ml GFS-medium and 20 ml sterile-filtered tap water. Plates were incubated in a µQuant spectrometer for three days at room temperature while the optical density of the wells' content was being measured at 600 nm every 2 hours, as suggested by Narendranath and Powers in 2004. The 96-well-plates were originally capped with enclosed lids, but the spectrometer's heat generation resulted in fogging of the lid. Changing the pattern from horizontal to vertical reduced the data loss, but was ultimately rejected in favor of membrane sealing in combination with the original pattern. Test runs on an Epoch spectrometer yielded slightly different results than µQuant runs, which led to this option being discarded as well and the application of breathe-easy membranes to close the plates. Fogging occurred in wells depicted in Figure 2. 100 ml of the 400c/µl-solution was also plated on YMPD medium as positive control.

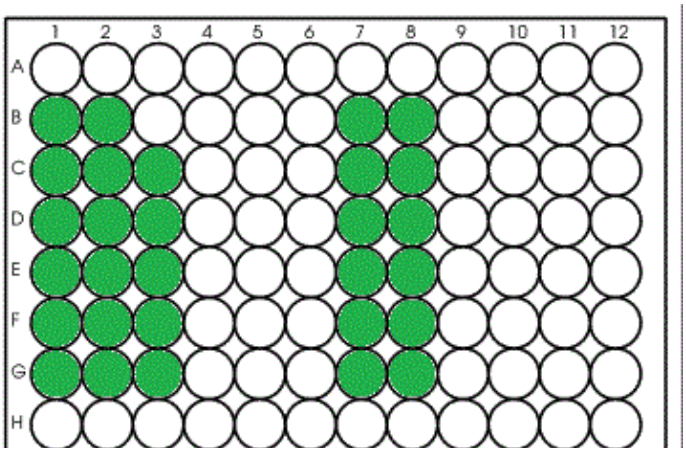

Figure 2: Lid fogging in µQuant spectrometer. Template © University of New South Wales.

After completion of the three-day-cycle, resulting data was saved in xml-format. The content of 4 randomized wells were taken as samples and transferred into 2 ml micro test tubes filled

with 0.3 mm glass pearls. These were centrifuged at 14.000 rpm for two minutes and stored at -80°C.

The following table lists *C. victoriae* strains successfully measured. Failed measurements, usually resulting from spectrometer cutoffs, are not listed here. The 'Date' tab refers to the start of the reading.

Table 3: List of completed *C. victoriae* measurements

Strain	**Origin**	**Date**
2A-28	*Taraxacum officinale*	30.11.2012
MO172	*Echium leucophaeum*	16.10.2012
MO174	*Echium leucophaeum*	19.10.2012
MO182	*Echium leucophaeum*	23.10.2012
MO184	*Echium leucophaeum*	26.10.2012
MO192	*Echium leucophaeum*	30.10.2012
MO193	*Echium leucophaeum*	12.10.2012
MO194	*Echium leucophaeum*	06.11.2012
MO229	*Echium strictum*	07.12.2012
MO230	*Echium strictum*	23.11.2012
MO245	*Echium leucophaeum*	27.11.2012
TSN486	Soil	11.12.2012
TSN529	Soil	14.12.2012

Due to positive results, various strains were tested on their ability to survive in 0%-sugar YNB-solution. All tested strains were incubated in this medium for several days and plated onto YMPD solid medium afterwards.

2.3 Statistical Analysis

Tables and graphs were created with Microsoft Excel 2007 (Microsoft Corporation). To compute box plots, raw data was calculated with SPSS. NMDA and regression plots were created with the freeware statistic program R and the Vegan package.

3. Results

Optical density measurement results of all strains are depicted in the appendix, tables 4 to 16. Mean values of each of the 15 repeats are displayed subsequently in the form of diagrams as a function of time.

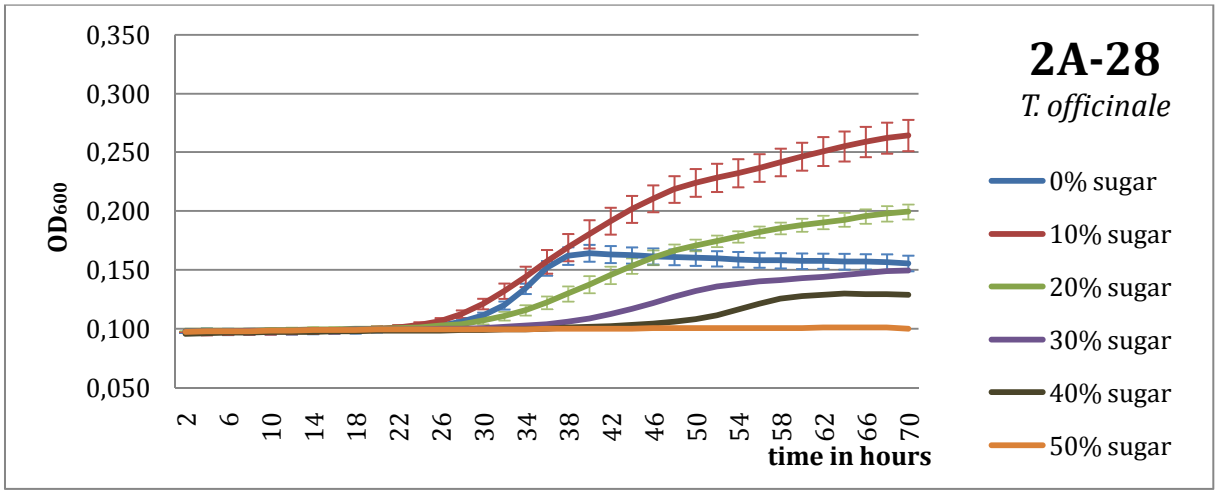

Figure 3: Growth pattern of strain 2A-28, with standard deviation added where applicable

Sample 2A-28 shows tolerance to all but the highest concentration of sugar. Cells of this strain also appear to multiply on YNB alone (0% sugar), albeit with a slightly sagging curve shape after apex.

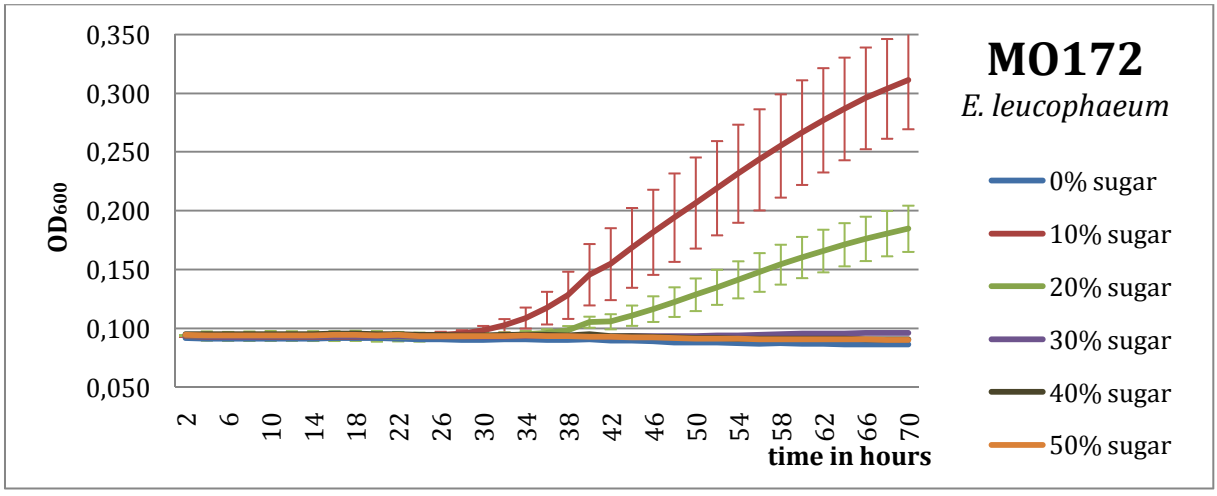

Figure 4: Growth pattern of strain MO172, with standard deviation added where applicable

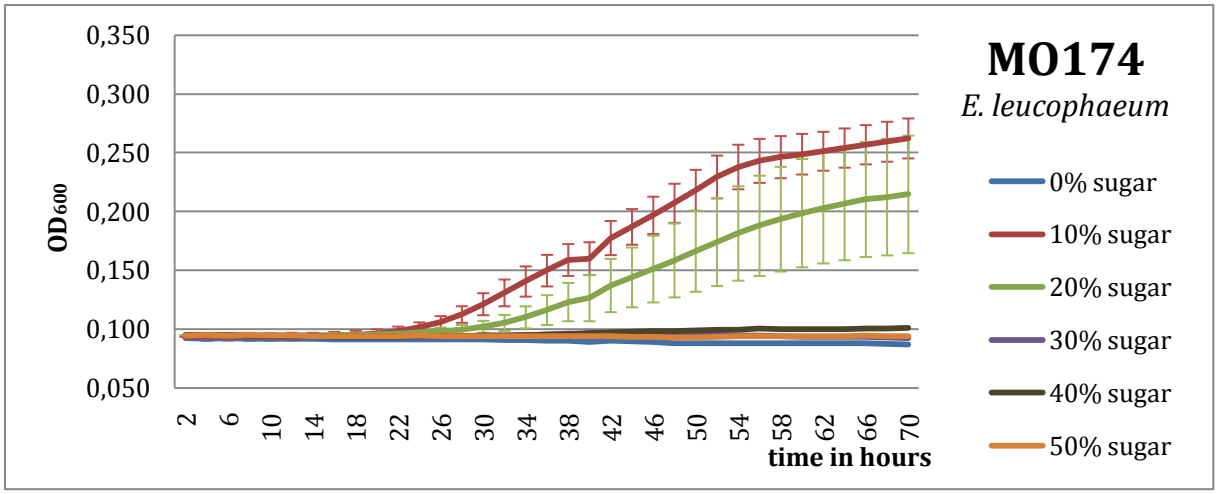

Figure 5: Growth pattern of strain MO174, with standard deviation added where applicable

Strain MO174 shows earlier increase of optical density correlating to cellular growth (at 22 hours after start for the culture cultivated in 10% GFS-medium and 26 hours in 20% GFS-medium) than strain MO172 (starting at 30 hours and 38 hours respectively). Also, while the 10% GFS curve shape of strain MO174 suggests a lower overall cell density than that of strain MO172 at 70 hours, the cell density of the 20% GFS culture appears to higher in MO174 than in MO172.

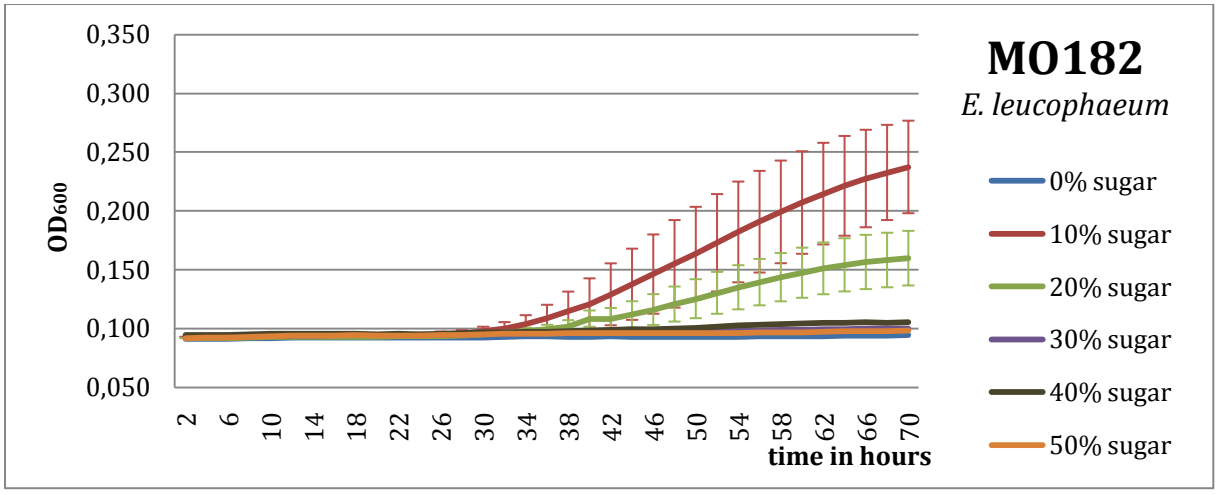

Figure 6: Growth pattern of strain MO182, with standard deviation added where applicable

MO182 shows a growth pattern similar to the two strains before, with the 10% GFS culture growing better than the one in 20% GFS-medium. Both maximum cell density levels appear to be lower in MO182.

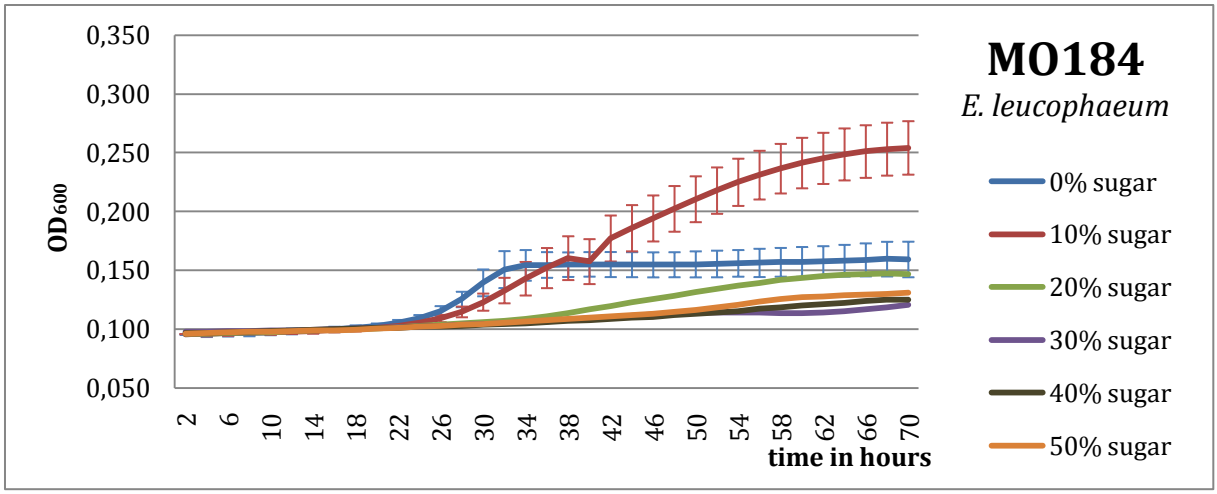

Figure 7: Growth pattern of strain MO184, with standard deviation added where applicable

The curve shape of MO184 in 10% GFS-medium shows the highest growth rate, while the growth rate of this strain in 20% GFS-medium is noticeably lower. The strain appears to grow in pure YNB-medium, albeit reaching a plateau after about 12 hours of growth. Also remarkable is an increase in optical density of all other cultures of this strain. Control smear suggested no contamination of the cultures.

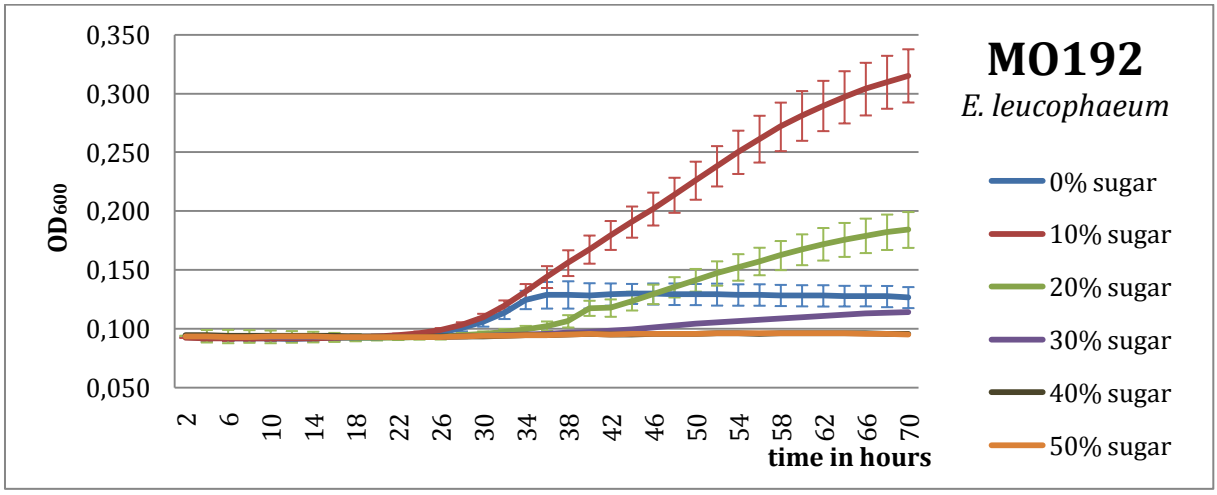

Figure 8: Growth pattern of strain MO192, with standard deviation added where applicable

Growth rate in strain MO192 is very high in the 10% GFS-medium culture and starts early after 22 hours. The culture in 20% GFS grows slower and begins several hours later. There is also some growth activity in 30% GFS as well as YNB-medium, but while the culture in the latter begins to grow early, it quickly reaches a stagnation point.

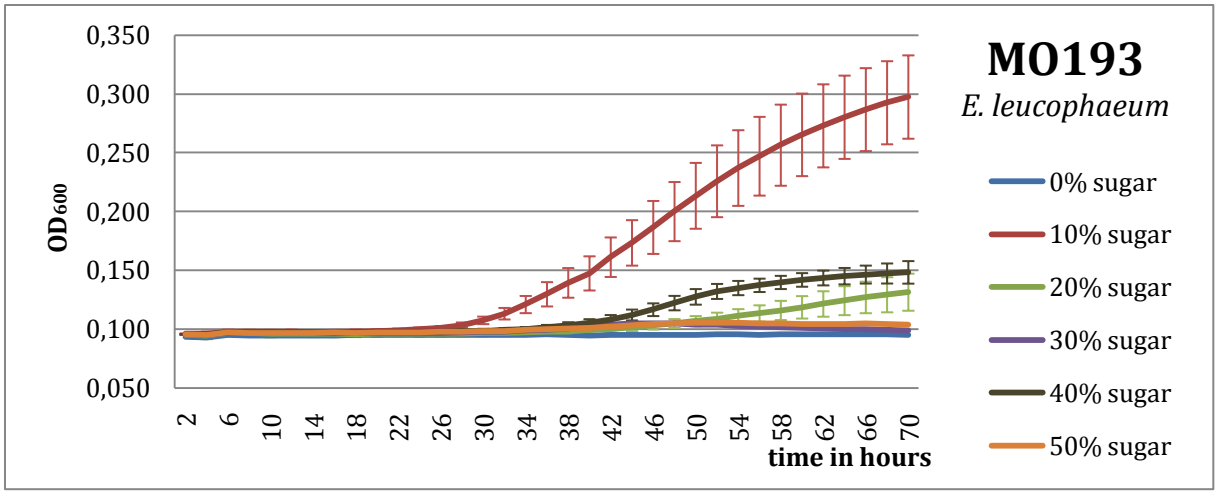

Figure 9: Growth pattern of strain MO193, with standard deviation added where applicable

Aside from a strong growth in 10% GFS-medium, strain MO193 shows little growth in 20% GFS-medium with a late start as well as slightly better growth in 40% GFS.

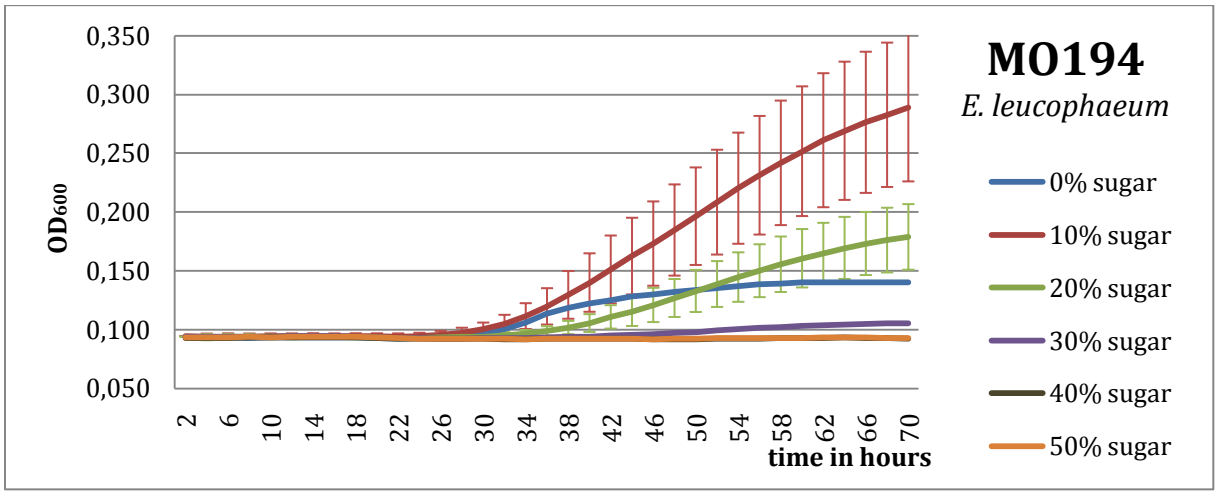

Figure 10: Growth pattern of strain MO194, with standard deviation added where applicable

Strain MO194's YNB-cultures shows a similar, yet flatter, progression to the culture from strain MO184, with a slower reached plateau phase. Again, no contamination could be detected. Cultures in 10% GFS-medium grow faster than those in 20% GFS, and don't reach saturation. A small increase in the optical density of cultures in 30% GFS-medium can be seen, but no sign of growth with other concentrations.

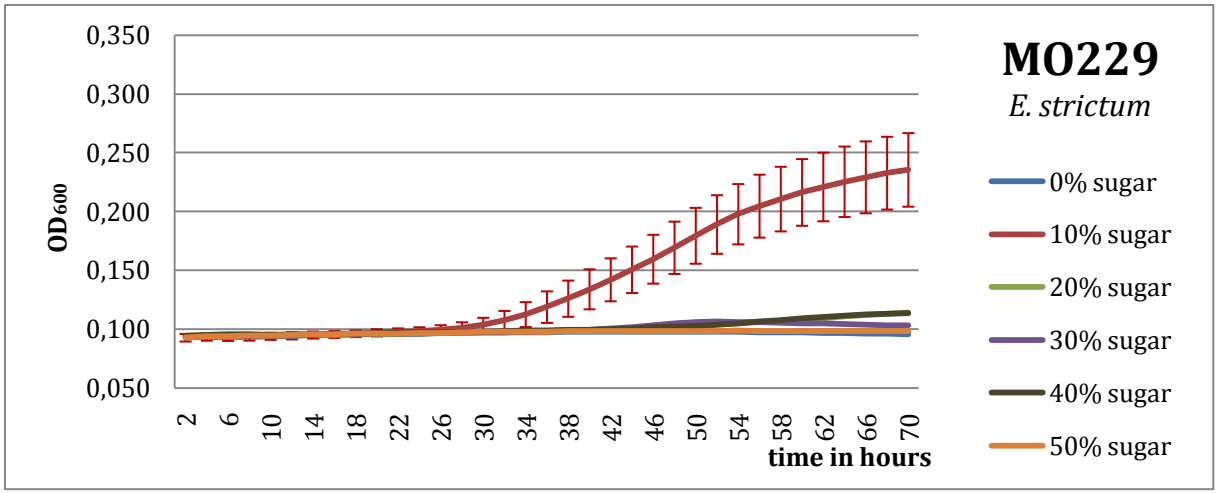

Figure 11: Growth pattern of strain MO229, with standard deviation added where applicable

Aside from minimal changes in the optical density of every other culture, strain MO220 shows growth activity only in 10% GFS-medium.

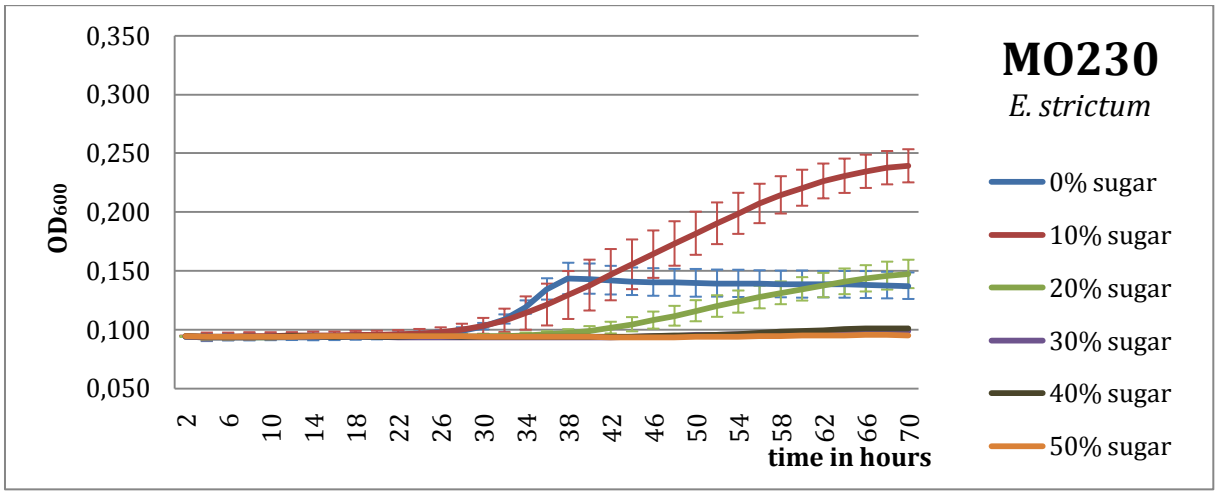

Figure 12: Growth pattern of strain MO230, with standard deviation added where applicable

In strain MO230, cultures incubated in 10% GFS-medium have the highest growth rates as well as a lower and more delayed rate for 20% GFS-medium cultures. Those cultivated in YNB-medium show a higher increase in cell density, but growth stagnates after approximately 10 hours. Again, no contamination within YNB-cultures could be detected.

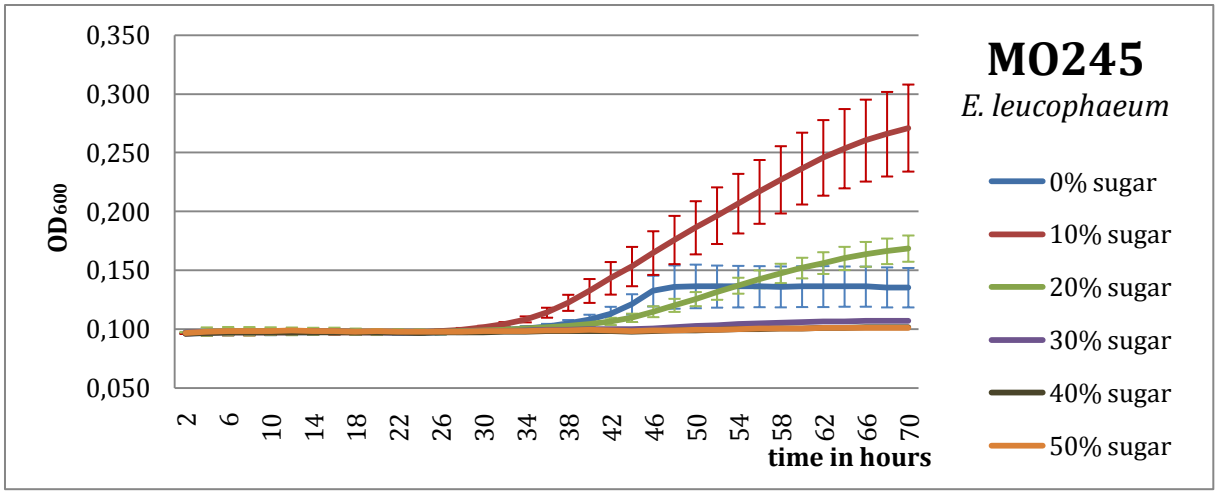

Figure 13: Growth pattern of strain MO245, with standard deviation added where applicable

Strain MO 245 is similar to MO230 in that 10% GFS-medium provides best conditions for growth, followed by 20% GFS-medium. The curve for YNB is similar to MO230, but begins several hours later and is less steep.

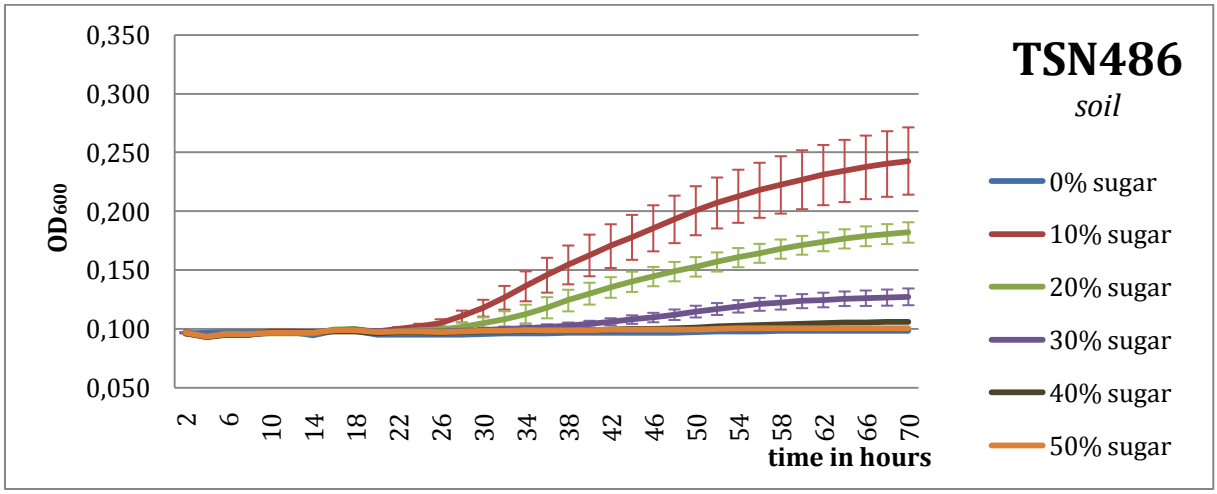

Figure 14: Growth pattern of strain TSN486, with standard deviation added where applicable

Strain TSN486, gathered from soil, shows strong increase in cell density when incubated in 10% GFS-medium, and about 40% less increase in 20% GFS. There is also noticeable, albeit weak, growth in 30% GFS-medium, but close to none in all other media.

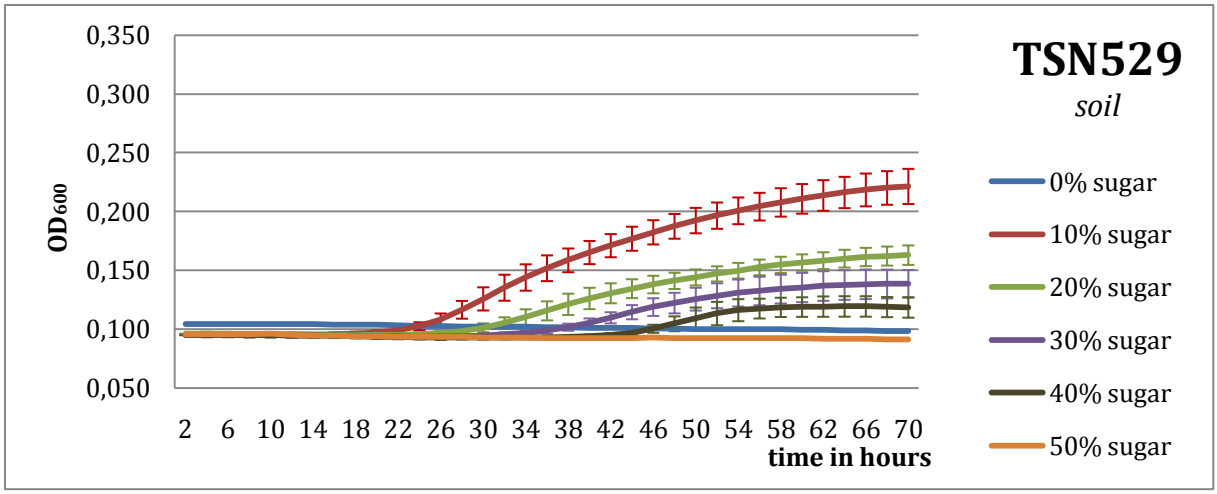

Figure 15: Growth pattern of strain TSN529, with standard deviation added where applicable

Both samples gathered from soils appear to tolerate higher concentrations of sugar, as they show growth activity in all but the highest concentration of 50% GFS. Growth is, however, inversely proportional to sugar concentration, and strain TSN529 as well as TSN486 show no sign of growth activity in YNB alone.

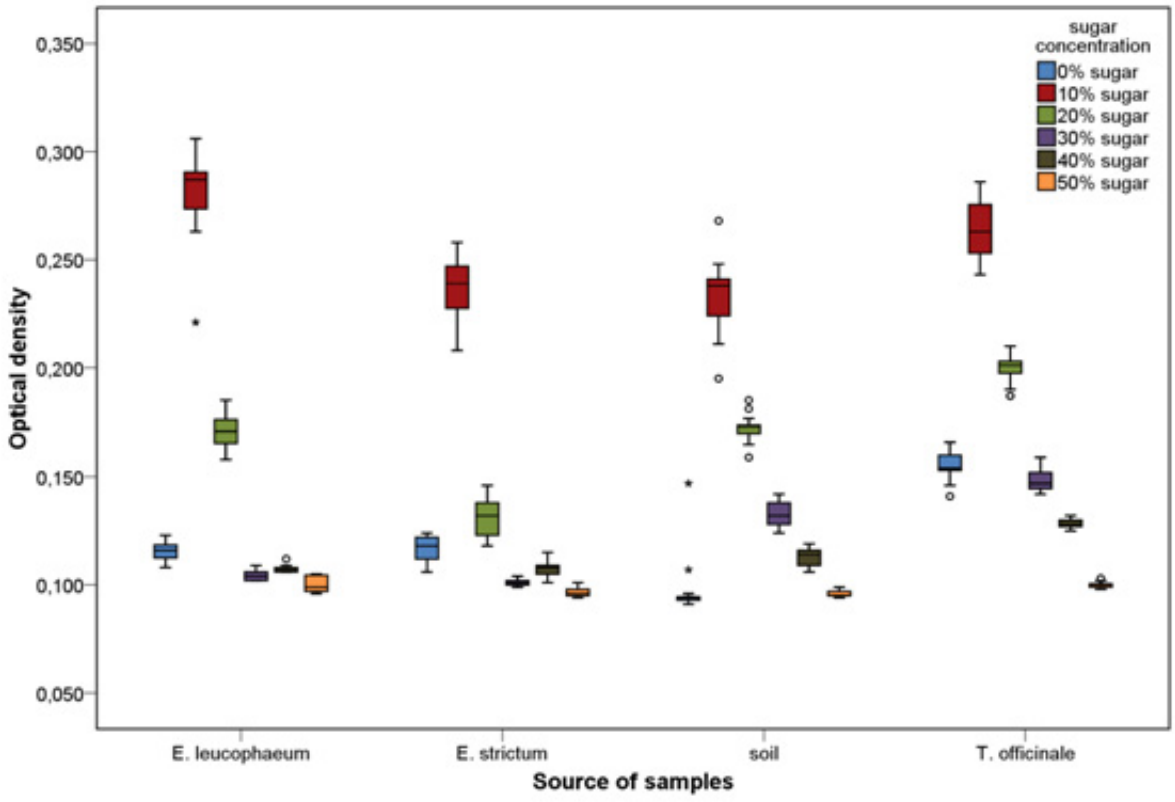

Figure 16: Box plot calculated from optical density values of the strains after 70 hours, summarized according to their source; From left to right: *Echium leucophaeum*, *Echium strictum* (both Tenerife, Spain), soil samples and *Taraxacum officinale* (both Bochum, Germany). High and low outliers are represented as asterisks and circles, respectively.

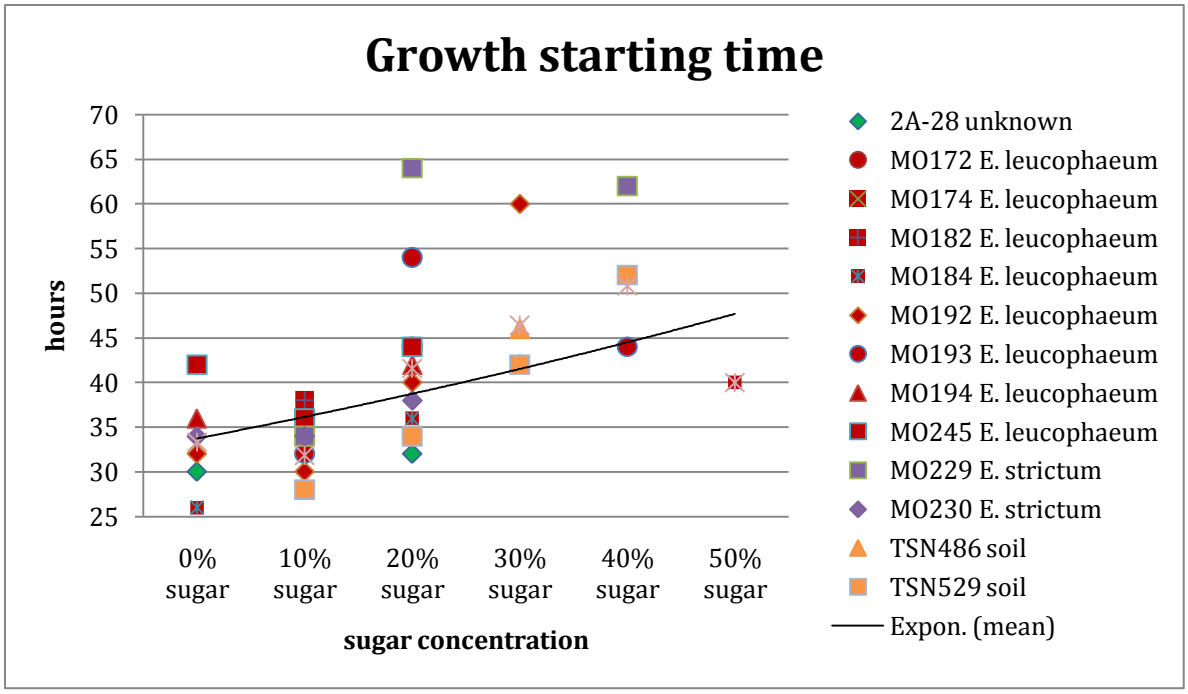

Figure 17: Incubation time when growth exceeded the original OD_{600} value by 10, with an added trend estimation line calculated from that starting means

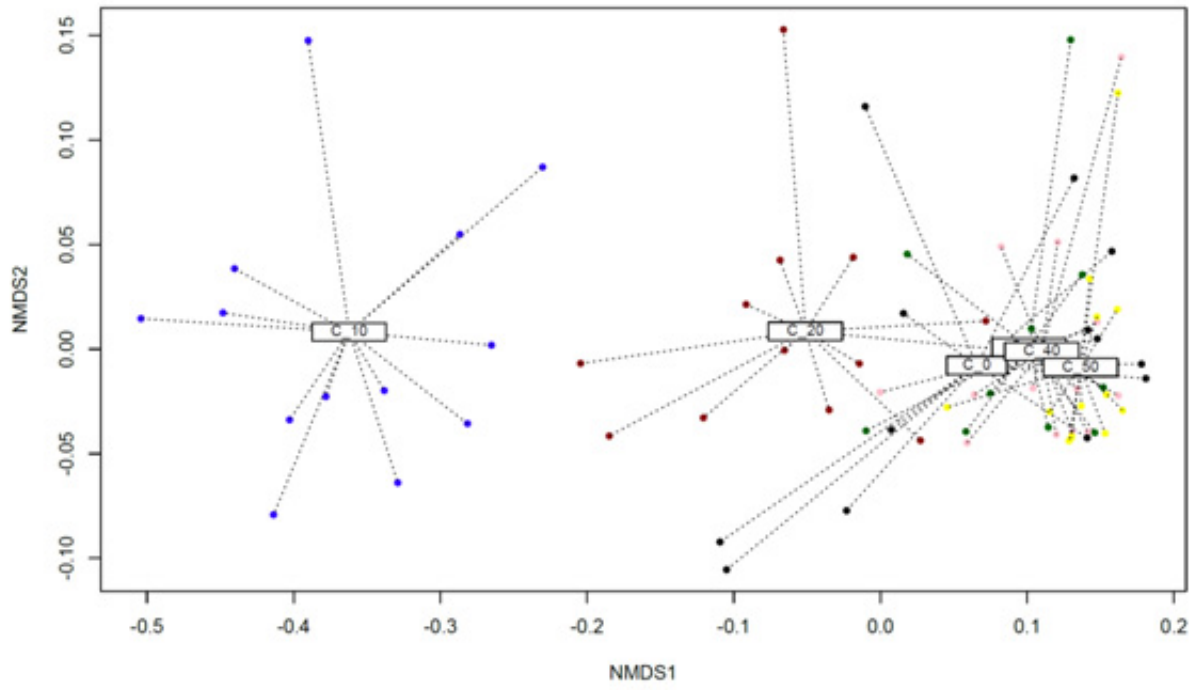

Figure 18: NMDS ordination plot calculated from measurements of all strains, displaying variation in growth depending on sugar concentration. Its stress plot can be found on p. 29, appendix, Figure 20.

4. Discussion

I. What are the responses of *Cryptococcus victoriae* to various different sugar concentrations?

All strains of *Cryptococcus victoriae*, with the exception of MO184 (see Figure 7), are very similar in their reaction to the highest concentration of GFS-medium of 50%, as no growth can be recognized by any of the tested strains. This is in accord with the findings of Thomas-Hall et al., 2002 (see Table 1). The increase in optical density is slight, however, and is likely a process artifact. Additionally, The NMDS plot (see Figure 18), calculated from all strains' replicates, illustrates that the growth of *Cryptococcus victoriae* deviates most from the others in 10% GFS-medium. Figures 3 to 15 also show that all tested strains grow best in 10% GFS-medium, which suggests that a concentration of around 10% sugar comes close to ideal environmental conditions.

While figures 3, 7, 8, 10, 12 and 13 show growth in pure YNB-media (0% GFS), figure 18 displays that the mean for the culture's optical density increase in YNB clusters with those of 30% to 50% GFS, in which the strains show little to no growth. Still, all samples incubated in YNB-medium for 3 days and plated on YMPD solid medium show that *C. victoriae* can tolerate an environment that is low on sugar and maintain a living population. As the mean for cell multiplication in 20% GFS-medium is located closer to that of 10% GFS-medium than YNB-medium, it is likely that ideal growth conditions are slightly higher than 10%, rather than lower. Figure 17 suggests that the cultures begin to grow later with increasing concentration of sugar. Cultures incubated in YNB-media appear to start growth earlier than in sugar-containing media, however their maximum cell density is below that of cultures from 10% and 20% GFS-medium, the latter with the exception of MO184 (see Figure 7).

All in all, tested strains *Cryptococcus victoriae* exhibit a reaction one would expect from a generalist species. Its preference of lower levels of sugar concentration and ability to sustain itself in medium very low on sugar contribute to its habitation of sites with extreme environmental conditions, where it has a clear advantage over more specialized species. In habitats with higher sugar concentration, however, *C. victoriae* appears to be disadvantaged compared to true specialist species like *Metschnikowia reukaufii*, which can proliferate in nectar with a sugar concentration up to at least 50% (Herrera et. al., 2012).

II. How much variation exists within a species?

Strains of *Cryptococcus victoriae* show variations when it comes to higher concentrations, some apparently being able to cope with the increased osmotic stress. Intraspecific variations are useful for a species that can be found seemingly spread randomly in various habitats. While habitat conditions may hinder the growth of some strains, others may yet prosper in them. The ordination plot in Figure 19 below also shows that growth behavior of the strains in is largely the same during the first 24 hours, after which it segregates. After approximately 60 hours, the growth patterns converge again.

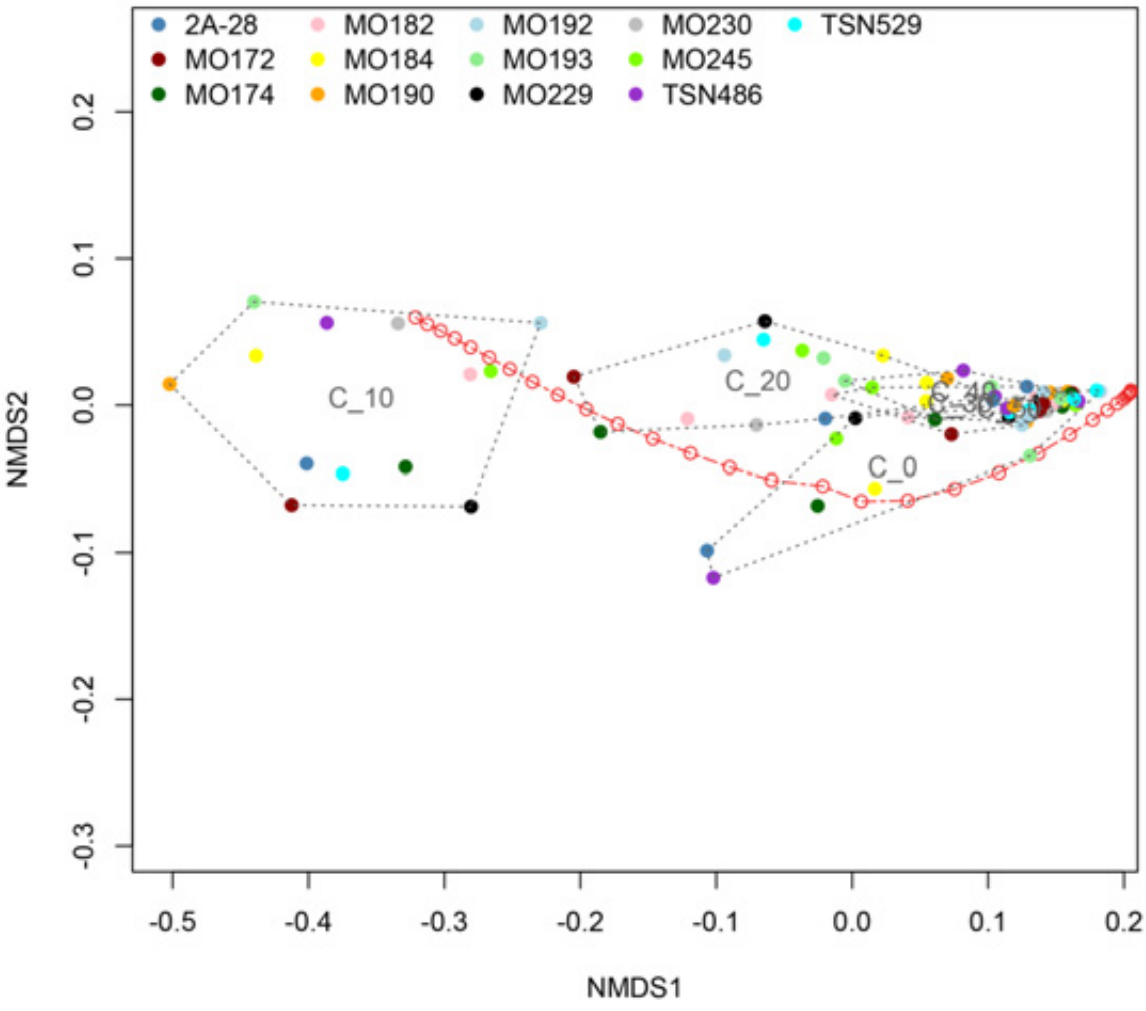

Figure 19: Variant NMDS ordination plot calculated from measurements of all strains, displaying variation in growth depending on sugar concentration. Filled circles represent values calculated from specific concentrations, while hollow circles were calculated from all measurements. All readings associated with a certain concentration are framed.

Few strains tested exhibit growth in medium with a concentration of 30 or 40%, with delayed growth and low maximum optical density values. Among these, strains 2A-28 (Figure 3), TSN529 (Figure 15) and MO184 (Figure 7) are the only ones which grow in both concentrations, the latter being the sole strain demonstrating growth in every concentration tested. Strains MO193 (Figure 9) and MO229 (Figure 11) grow in 40% GFS-medium, but not in 30% GFS-medium, which is likely a measurement artifact.

All strains of *Cryptococcus victoriae* exhibit highest growth rates in 10% GFS-medium (see Figure 19) and close to no growth in 50% GFS-medium (see Figure 16), but variations can be discerned. Several strains exhibit an increase in cell number when incubated in YNB-medium, namely 2A-28 (Figure 3), MO184 (Figure 7), MO192 (Figure 8), MO194 (Figure 10), MO230 (Figure 12) as well as MO245 (Figure 13). These strains' starting times also vary considerably, as can be seen in figure 17, ranging from 26 hours after beginning of measurements in MO184 to 42 hours in MO245.

While all strains grow in 20% GFS-medium, maximum optical density at 70 hours varies strongly among the strains, and the starting time of growth ranges from 32 hours in 2A-28 (Figure 3) to 64 hours in MO229 (Figure 11). In contrast to the more tightly clustered starting points when incubated in 10% GFS-medium (see figure 17), it can be deduced that *Cryptococcus victoriae* strains vary in their acceptance of medium with a sugar concentration of around 20%.

III. Can a habitat-induced predisposition be discerned?

Cryptococcus victoriae strains gathered from *E. leucophaeum* show slightly greater average increase in cell number in 10% GFS-medium than those from *T. officinale* and a more distinct increase compared to those from *E. strictum* and soil. This coincides with the fact that *E. leucophaeum* has a lower nectar concentration (10-30%) than *E. strictum* (50-70%), but does neither explain why the strains collected from soil grow comparatively little nor why those from the epiphyll of *T. officinale* grow almost as well. As a concentration of 10% sugar comes close to ideal nourishment conditions for *C. victoriae*, it would seem that the source from where the strains stem has no influence on its growth behavior in 10% GFS-medium.

Growth in 20% GFS-medium follows a similar pattern, albeit with lower overall maxima. Samples from *E. strictum* grow worse than samples from *E. leucophaeum*, as would be expected considering the higher sugar concentration of the former plant's nectar. *C. victoriae*

strains collected from *E. leucophaeum* could, however, be expected to grow better in 20% GFS-media than in 10%, as 20% would fall neatly into the mean nectar concentration of *E. leucophaeum.* This suggests that these strains develop no predisposition through their former habitat. The strain collected from *T. officinale* shows the highest growth rate in 20% GFS-medium, despite the expected low amount of sugar of its host plant's epiphyll. This, too, shows that no habitat-induced predisposition seems to be present.

Another indicator is that the soil samples of *C. victoriae* grow worse in pure YNB-medium than samples of every other group, despite the fact that soil usually contains very little sugar, with concentrations of around 0.2% (Lowe, 1978), of which 80% is glucose. In contrast, the sample from *T. officinale* grows best of all samples in YNB-medium, which could be expected. Additionally, almost no samples, including strains from *E. strictum* with a nectar concentration of up to 70%, grow in 50% GFS-medium, which would be likely if a habitat-induced predisposition existed.

An untested possibility for habitat-induced predisposition is a resistance to antimicrobial substances many flowering plants are known to secrete into their nectar (Lawton et. al., 1993). In this, an adaption to higher sugar concentrations might have been forgone in favor of higher resistances to growth-inhibiting additives. It is also known that generalist fungi species could be less affected by plant-secreted deterrents than specialists (Marak et al., 2002). GFS-media used for this thesis did not contain any such ingredients, and the effect of a potential predisposition could not have been verified. Nectar specialized yeasts like *Metschnikowia* also adapted to the isolated nature of their habitat, as they are able to utilize pollinators as means of transportation between flowers (Hong et al., 2003) and may even overwinter inside their intestines (Herrera et al., 2010). Even though it is possible for similar abilities to exist, no proof has yet been found of such adaption among *Cryptococcus*. Plant-pollinator-interaction is also influenced by amino-acids secreted into the nectar (Baker, 1975). It is possible that variations in the contained amino acids of different plants' nectar influences growth behavior of *C. victoriae* as well.

IV. Do specialists for sugar-based habitats exist among *C. victoriae*?

Among the strains tested for this thesis, no specialist strains for sugar-based habitats could be found among *C. victoriae*, meaning no strain excels in highly concentrated medium. Only 10% GFS-medium and, to a lesser extent, 20% GFS-medium, support the highest rate of

growth. Figure 18 shows that the maximum optical density values of strains for 0%, 30%, 40% and 50% GFS-medium cluster together, while only values for 10% and 20% are estimated with less proximity, supporting what can be deduced from figures 3 to 15. Start of growth also appears to be delayed with increasing sugar concentration of the surrounding medium (see figure 17). That being said, strains of *Cryptococcus victoriae* are apparently able to sustain themselves in sugar concentrations higher and lower than its preferred optimum. This ability is mandatory for survival in nectar of flowering plants, as its sugar concentration fluctuates due to temperature (Baker, 1975 & Calder, 1979) and consumption (Nicholson, 1998), and resident yeasts need to withstand these effects until habitat conditions improve again, e.g. once the nectar concentration is restored by the host. *C. victoriae* is also able to grow in media containing little to no sugar, increasing its cell division activity when sufficient nourishment is available, as long as the osmotic pressure does not exceed a limit reserved for true specialists, like *Metschnikowia*. Yeasts growing well in low carbon environments exist, but they pay for this ability with reduced fitness in habitats where sugar is more abundant (Wenger et. al, 2011). *C. victoriae* displays neither specialization for high nor low concentrations of sugar. It rather tolerates unfavorable conditions with reduced metabolism until its habitat improves.

5. Abstract

Cryptococcus victoriae is a species of yeasts that can be found ubiquitous in and on plants, animals, soil, air and water, with a dispersal area ranging temperate regions to extreme sites like glaciers, ice deserts or dry deserts. It is also able to assimilate various carbohydrate compounds, several types of sugar, sugar alcohols or starch among them. *C. victoriae* is therefore usually described as a generalist species of fungi, being able to cope with various different habitat conditions.

Among the places it can be found is the nectar of flowering plants. This habitat provides unfavorable conditions due to limited space, temporal and spatial fragmentation due to the flowering cycles as well as high osmotic stress caused by the potentially high sugar concentrations. Yet, *C. victoriae* seems to be more abundant in nectar than specialist species like *Metschnikowia reukaufii*.

Tests of *C. victoriae* in sugar-based liquid media, labeled GFS-medium for its composition of glucose, fructose and sucrose in equal ratio, have shown that this species grows best in 10% sugar and reasonably well in 20% sugar. Several tested strains were also able to grow, and therefore survive, in sugar concentrations of up to 40%, in one case even 50%. More than half of the tested strains also showed growth activity when cultivated in Yeast nitrogen base medium with no added sugar. Incubation of these strains on YMPD solid medium afterwards revealed that they were able to cope with a lack of sufficient nutrition and resume heightened proliferation activity once subject to more favorable conditions.

The tests also demonstrated that *C. victoriae* does not show habitat-induced predispositions. It is a true generalist species, and while some strains are able to better deal with conditions others could not, they do not develop specialization towards former habitats.

6. References

Baddley, J. W., Perfect, J. R. et al. (2008). Pulmonary cryptococcosis in patients without HIV infection: factors associated with disseminated disease. *Eur. J. Clin. Microbiol. Infect. Dis.* **27,** 937-942.

Baker, H. G. (1975). Sugar concentrations in Nectars of Hummingbird Flowers. *Biotropica*. **7.** 37-41.

Baum, G. L., Artis, D. (1965). Isolation of Fungi from Judean desert soil. *Mycopat. et mycol. applicata* **29,** 350-354.

Benham, Rhoda W. (**1956**). The genus Cryptococcus. *Bacteriol. Rev.* **20,** 189-199.

Brizzio, S., Turchetti, B., de García, V., Libkind, D., Buzzini, P., van Broock, M. (2007). Extracellular enzymatic activities of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia (Argentina). *Canad. J. Microbiol.* **53(4),** 519-525.

Calder, W. A. III (1979). On the temperature-dependency of optimal nectar concentration for birds. *J. Theor. Biol.* **78.** 185-196.

Corbet, S. A., Delfosse, E.S. (1984). Honeybees and the nectar of *Echium plantagineum* L. in southern Australia. *Austr. J. Ecol.* **9,** 125-139.

Ecroyd, C.E., Franich, R.A., Kroese, H.W. and Steward, D. (1995). Volatile constituents of *Dactylanthus taylorii* flower nectar in relation to flower pollination and browsing by animals. *Phytochemistry* **40,** 1387–1389.

Fell, J.W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. (2000). Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int. J. Syst. Evol. Microbiol.* **50,** 1351–1371.

de Garcia, V., Brizzio, S., Russo, C. A., Teun Boekhout, R., Theelen, B., Libkind, D., van Broock, M. (2010). *Cryptococcus spencermartinsiae* sp. nov., a basidiomycetous yeast isolated from glacial waters and apple fruits. *Int. J. Syst. Evol. Microbiol.* **60,** 707–711.

de Garcia, V., Zalar, P., Brizzio, S., Gunde-Cimerman N., van Broock, M. (2012). *Cryptococcus* species (Tremellales) from glacial biomes in the southern (Patagonia) and northern (Svalbard) hemispheres. *FEMS Microbiol. Ecol.* **82,** 523–539.

Herrera, C.M., Canto, A., Pozo, M. I., Bazaga, P. (2010). Inhospitable sweetness: nectar filtering of pollinator-borne inocula leads to impoverished, phylogeneically clustered yeast communities. *Proc. Biol. Sci*. **277,** 747-754.

Herrera, C. M., García, I. M., Pérez, R. (2008). Invisible floral larcenies: microbial communities degrade floral nectar of bumblebee-pollinated plants. *Ecology* **89,** 2369-2376.

Herrera, C. M., Pozo, M. I. (2012). Nectar yeasts warm the flowers of a winter-blooming plant. *Proc. Biol. Sci.* **277,** 1827-1834.

Herrera, C. M., de Vega, C., Canto, A., Pozo, M. I. (2009). Yeasts in floral nectar: a quantitative survey. *Ann. Bot.* **103,** 1415-1423.

Hong, S. G., Bae1, K. S., Herzberg, M., Titze, A., Lachance, M. A. (2003). C*andida kunwiensis* sp. nov., a yeast associated with flowers and bumblebees. *Int. J. Syst. Evol. Microbiol.* **53,** 367-372.

Kabir, G. P., Belisle, M., Fukami, T. (2011). Phylogenetic relatedness predicts priority effects in nectar yeast communities. *Proc. R. Soc. B.* **279,** 749-758.

Kaewwichian, R., Yongmanitchai, W., Kawasaki, H., Limtong, S. (2012). *Metschnikowia saccharicola* sp. nov. and *Metschnikowia lopburiensis* sp. nov., two novel yeast species isolated from phylloplane in Thailand. *Antonie van Leeuwenhoek* **102,**743–751.

Kauffman, C.A., Goldman, L. (2007). Cryptococcosis. *Cecil Medicine*. **23,** ch. 357.

Kreger-van Rij, N. J. W. (1964). The genus Cryptococcus. *Ann. Soc. Belge Méd trop.* **44,** 601-610.

Kwon-Chung K.J., Polacheck I., and Bennett J.E. (1982). Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (Serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (Serotypes B and C). *J. Clin. Microbiol.* **15(3),** 535-537.

Kwon-Chung, K.J. & J.E. Bennett (1992). Medical Mycology. *Rev. Inst. Med. trop. S. Paulo.* **34,** 866 pp.

Lachance, M. A., Boekhout, T., Scorzetti, G., Fell, J. W. & Kurtzman, C. P. (2011). Candida. *The Yeasts, a Taxonomic Study, 5th ed.*, pp. 987–1278. Edited by C. P. Kurtzman, J. W. Fell & T. Boekhout. Amsterdam: Elsevier Science.

Lawton, R. O., Alexander, L. D., Setzer, W. N., Byler, K. G. (1993). Floral essential oil of *Guettarda poasana* inhibits yeast growth. *Biotropica* **25.** 483–486.

Lowe, L. E. (1978). Carbohydrates in soil. *Soil Organic Matter*, Elsevier, Amsterdam, pp. 1–64.

Marak, H. B., Biere, A., van Damme, J. M. M. (2002). Two herbivore-deterrent iridoid glycosides reducethe in-vitro growth of a specialist but not of a generalistpathogenic fungus of *Plantago lanceolata* L. *Chemoecology* **12,** 185-192.

Mendonça-Hagler, L. C. (1993). Phylogeny of *Metschnikowia* species estimated from partial rRNA sequences. *Int. J. Syst. Bacteriol.* **43,** 368 pp.

Metschnikoff, E. (1884). Über eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre uber den Kampf der Phagocyten gegen Krankheitserreger. *Arch. Pathol. Anat. Physiol. R. Virchow.* **96,** 177-195.

Miller, M. W., Barker, E. R., Pitt, J. I. (1967). Ascospore numbers in *Metschnikowia*. *J. Bacteriol.* **94,** 258-259.

Montes, M. J., Belloch, C., Galiana, M., Garcia, M. D., Andre! s, C., Ferrer, S., Torres-Rodriguez, J. M. & Guinea, J. (1999). Polyphasic taxonomy of a novel yeast isolated from Antarctic environment; description of *Cryptococcus victoriae* sp. nov. *Syst. Appl. Microbiol.* **22,** 97±105.

Narendranath, N. V., & Power, R. (2004). Relationship netween pH and Medium Dissolved Solids in Terms of Growth and Metabolism of Lactobacili and *Saccharomyces cerevisiae* during Ethanol Production. *Appl. and Env. Microbiol.* **71,** 2239-2243.

Nicolson, S. W. (1998). The Importance of Osmosis in Nectar Secretion and its Consumption by Insects. *Amer. Zool.* **38.** 418-425.

Nicolson, S. W., Nepi, M., Pacini, E. (2007). Nectaries and Nectars. *Springer Publications* p. 1-18.

Pitt, J. I., Miller, M. W. (1968). Sporulation in *Candida pulcherrima, Candida reukaufii* and *Clamydozyma* species: Their relationships with *Metschnikowia*. *Mycologia* **60,** 663-685.

Pitt, J. I., Miller, M. W. (1970). Speciation in the yeast genus *Metschnikowia*. *Antonie van Leeuwenhoek* **36,** 357-381.

Pozo, M. I., Lachance, M. A., Herrera, C. M. (2012). Nectar yeasts of two southern Spanish plants: the roles of immigration and physiological traits in community assembly. *FEMS Microbiol. Ecol.* **80,** 281-293.

Shivaji, S., Prasad, G.S. (2009). Antarctic yeasts: biodiversity and potential applications. *Yeast Biotechnology: Diversity and Application* (Satyanarayana T & Kunze G, eds), pp. 3–18. Springer Science + Business Media B.V., Netherlands.

Sneller, M. R., Swatek, F. W. (1974). Distribution of the genus *Cryptococcus* in Southern California soils. *Sabouraudia* **12,** 46-53.

Szabo, T. I. (1984). Nectar secretion in dandelion. *J. Apicult. Res.* **23,** 204-208.

Thomas-Hall, Skye, Watson, K., Scorzetti, G. *(2002*). *Cryptococcus statzelliae* sp. nov. and three novel strains of *Cryptococcus victoriae*, yeasts isolated from Antarctic soils. *Int. J. Syst. Evol. Microbiol*. **52**, 2303–2308.

de Vega, G., Guzmán, B., Lachance, M. A., Steenhuisen, A. L., Johnson, S. D., Herrera, C. M. (2012). *Metschnikowia proteae* sp. nov., a nectarivorous insect-associated yeast species from Africa. . *Int. J. Syst. Evol. Microbiol*. **62,** 2538–2545.

Vishniac, H. S. (1985). *Cryptococcus friedmannii*, a New Species of Yeast from the Antarctic. *Mycologia* **77(1),** 149-153.

Vishniac, H. S. (1985). *Cryptococcus socialis* sp. nov. and *Cryptococcus consortionis* sp. nov., Antarctic Basidioblastomycetes. *Int. J. Syst. Evol. Microbiol*. **35,** 119-122.

Vuillemin, Paul (1901). *Cryptococcus neoformans* (San Felice). *Rev. Gén. Sci. Pures Appl.* **12,** 747-750.

Wenger, W. J., Piotrowski, J., Nagarajan, S., Chiotti, K., Sherlock, G., Rosenzweig, F. (2011). Hunger Artists: Yeast Adapted to Carbon Limitation Show Trade-Offs under Carbon Sufficiency. *PLoS Genet*. **7.** e1002202.

7. Appendix

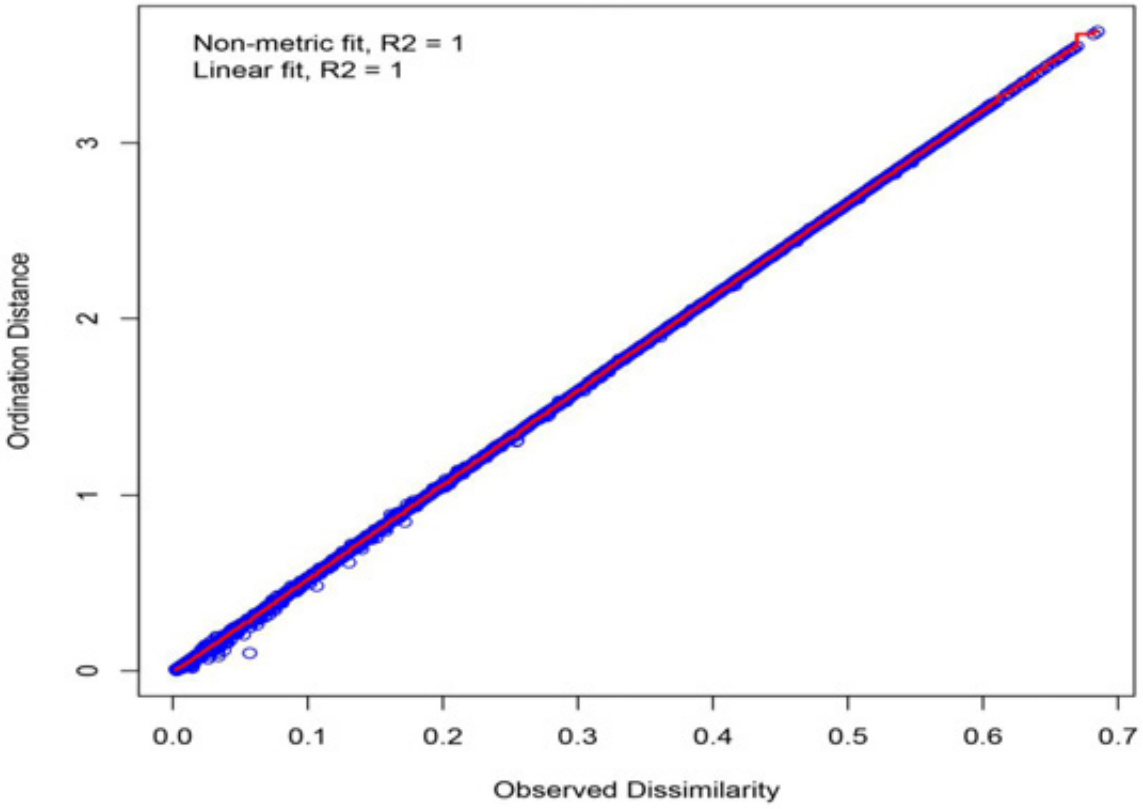

Figure 20: Stressplot

Table 4: Mean optical density values of strain 2A-28, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,099	0,097	0,098	0,097	0,097	0,097	0,098	0,098	0,098	0,098	0,099	0,100
10% sugar	0,098	0,097	0,097	0,097	0,097	0,098	0,098	0,098	0,098	0,099	0,100	0,101
20% sugar	0,098	0,098	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,100	0,100	0,101
30% sugar	0,098	0,097	0,098	0,098	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,099
40% sugar	0,095	0,096	0,096	0,096	0,097	0,097	0,097	0,097	0,098	0,098	0,098	0,098
50% sugar	0,096	0,097	0,097	0,098	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,099
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,101	0,103	0,106	0,111	0,120	0,134	0,151	0,162	0,164	0,163	0,162	0,162
10% sugar	0,103	0,107	0,112	0,121	0,132	0,144	0,157	0,169	0,180	0,192	0,202	0,211
20% sugar	0,101	0,102	0,104	0,107	0,111	0,116	0,122	0,130	0,138	0,145	0,153	0,160
30% sugar	0,100	0,100	0,100	0,101	0,101	0,102	0,104	0,106	0,109	0,112	0,117	0,122
40% sugar	0,098	0,098	0,099	0,099	0,099	0,099	0,100	0,101	0,101	0,102	0,103	0,104
50% sugar	0,099	0,099	0,099	0,099	0,099	0,100	0,100	0,100	0,100	0,100	0,100	0,100
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,161	0,160	0,160	0,159	0,158	0,158	0,157	0,157	0,157	0,157	0,157	0,007
10% sugar	0,219	0,224	0,228	0,232	0,237	0,242	0,246	0,251	0,255	0,259	0,262	0,013
20% sugar	0,166	0,171	0,174	0,178	0,182	0,185	0,188	0,190	0,193	0,195	0,198	0,007
30% sugar	0,127	0,132	0,136	0,138	0,140	0,141	0,143	0,144	0,146	0,147	0,149	0,005
40% sugar	0,106	0,108	0,111	0,116	0,121	0,125	0,128	0,129	0,130	0,130	0,129	0,002
50% sugar	0,100	0,100	0,100	0,101	0,100	0,100	0,101	0,101	0,101	0,101	0,101	0,002

Table 5: Mean optical density values of strain MO172, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,092	0,091	0,091	0,091	0,091	0,091	0,091	0,092	0,091	0,091	0,091	0,090
10% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
20% sugar	0,094	0,094	0,093	0,093	0,094	0,093	0,093	0,093	0,094	0,093	0,093	0,093
30% sugar	0,094	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,094	0,093	0,093
40% sugar	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,094
50% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,091	0,090	0,090	0,091	0,091	0,090	0,090	0,090	0,089	0,089	0,089	0,088
10% sugar	0,094	0,096	0,098	0,102	0,109	0,117	0,128	0,146	0,155	0,169	0,182	0,194
20% sugar	0,093	0,093	0,093	0,094	0,095	0,096	0,098	0,105	0,106	0,111	0,116	0,122
30% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
40% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,095	0,093	0,093	0,093	0,092
50% sugar	0,094	0,093	0,093	0,094	0,094	0,093	0,093	0,093	0,093	0,092	0,092	0,092
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,088	0,088	0,087	0,087	0,087	0,087	0,087	0,086	0,086	0,086	0,086	0,002
10% sugar	0,207	0,219	0,232	0,243	0,255	0,267	0,277	0,287	0,296	0,304	0,311	0,041
20% sugar	0,129	0,135	0,141	0,148	0,154	0,160	0,166	0,171	0,176	0,181	0,185	0,020
30% sugar	0,093	0,094	0,094	0,094	0,095	0,095	0,096	0,096	0,096	0,096	0,096	0,005
40% sugar	0,092	0,092	0,091	0,091	0,091	0,091	0,091	0,091	0,091	0,091	0,091	0,004
50% sugar	0,091	0,091	0,091	0,091	0,091	0,091	0,091	0,090	0,090	0,090	0,090	0,002

Table 6: Mean optical density values of strain MO174, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,092	0,092	0,092	0,092	0,092	0,092	0,091	0,091	0,091	0,091	0,091	0,091
10% sugar	0,094	0,093	0,093	0,093	0,093	0,094	0,094	0,094	0,095	0,096	0,098	0,101
20% sugar	0,095	0,094	0,094	0,095	0,095	0,095	0,094	0,095	0,095	0,095	0,096	0,097
30% sugar	0,095	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
40% sugar	0,095	0,095	0,095	0,094	0,094	0,094	0,094	0,094	0,093	0,093	0,093	0,094
50% sugar	0,095	0,095	0,095	0,095	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,091	0,091	0,091	0,091	0,090	0,090	0,090	0,089	0,090	0,089	0,089	0,088
10% sugar	0,106	0,112	0,121	0,131	0,141	0,150	0,159	0,160	0,177	0,187	0,197	0,207
20% sugar	0,098	0,100	0,102	0,105	0,110	0,116	0,123	0,126	0,137	0,144	0,151	0,158
30% sugar	0,094	0,094	0,094	0,094	0,095	0,095	0,096	0,096	0,097	0,096	0,096	0,096
40% sugar	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,096	0,097	0,097	0,098	0,098
50% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093	0,093
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,088	0,088	0,088	0,088	0,088	0,088	0,088	0,088	0,088	0,087	0,087	0,001
10% sugar	0,218	0,229	0,238	0,243	0,246	0,249	0,251	0,254	0,257	0,259	0,262	0,017
20% sugar	0,166	0,174	0,181	0,188	0,194	0,199	0,203	0,207	0,210	0,212	0,215	0,051
30% sugar	0,095	0,095	0,094	0,094	0,094	0,093	0,093	0,093	0,093	0,093	0,092	0,002
40% sugar	0,099	0,099	0,100	0,100	0,100	0,100	0,100	0,100	0,100	0,100	0,101	0,003
50% sugar	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,004

Table 7: Mean optical density values of strain MO182, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,091	0,091	0,091	0,091	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092
10% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,094	0,094	0,094	0,094
20% sugar	0,092	0,092	0,092	0,092	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
30% sugar	0,093	0,093	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,095
40% sugar	0,095	0,094	0,095	0,095	0,095	0,095	0,096	0,096	0,096	0,095	0,096	0,095
50% sugar	0,092	0,092	0,092	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,092	0,092	0,092	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
10% sugar	0,095	0,096	0,098	0,100	0,104	0,108	0,115	0,121	0,129	0,137	0,146	0,155
20% sugar	0,094	0,094	0,095	0,096	0,098	0,099	0,102	0,108	0,108	0,112	0,116	0,121
30% sugar	0,095	0,095	0,095	0,096	0,096	0,096	0,096	0,096	0,097	0,097	0,097	0,097
40% sugar	0,095	0,096	0,096	0,097	0,097	0,097	0,098	0,098	0,099	0,099	0,099	0,100
50% sugar	0,094	0,094	0,095	0,095	0,096	0,095	0,096	0,096	0,096	0,096	0,096	0,096
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,001
10% sugar	0,164	0,173	0,182	0,191	0,199	0,207	0,214	0,221	0,227	0,232	0,237	0,038
20% sugar	0,125	0,130	0,135	0,139	0,143	0,147	0,151	0,154	0,156	0,158	0,160	0,023
30% sugar	0,097	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,100	0,100	0,100	0,003
40% sugar	0,101	0,102	0,103	0,103	0,104	0,104	0,105	0,105	0,105	0,105	0,105	0,005
50% sugar	0,096	0,096	0,096	0,097	0,097	0,097	0,097	0,097	0,098	0,098	0,098	0,002

Table 8: Mean optical density values of strain MO184, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,095	0,096	0,096	0,097	0,097	0,098	0,099	0,100	0,101	0,103	0,105	0,109
10% sugar	0,096	0,096	0,096	0,097	0,097	0,098	0,098	0,099	0,100	0,101	0,103	0,105
20% sugar	0,096	0,096	0,097	0,097	0,097	0,098	0,099	0,099	0,100	0,101	0,101	0,102
30% sugar	0,098	0,098	0,098	0,098	0,099	0,099	0,099	0,100	0,101	0,101	0,102	0,102
40% sugar	0,097	0,097	0,097	0,098	0,098	0,099	0,099	0,100	0,100	0,101	0,101	0,102
50% sugar	0,096	0,096	0,097	0,098	0,098	0,098	0,099	0,099	0,100	0,100	0,101	0,102
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,115	0,125	0,139	0,151	0,154	0,155	0,155	0,155	0,155	0,155	0,155	0,155
10% sugar	0,109	0,115	0,123	0,133	0,143	0,152	0,160	0,157	0,177	0,186	0,194	0,202
20% sugar	0,103	0,105	0,106	0,107	0,109	0,111	0,113	0,117	0,120	0,123	0,126	0,128
30% sugar	0,103	0,104	0,104	0,105	0,106	0,107	0,108	0,109	0,110	0,111	0,112	0,113
40% sugar	0,102	0,103	0,104	0,104	0,105	0,106	0,107	0,108	0,109	0,110	0,110	0,112
50% sugar	0,103	0,104	0,104	0,105	0,106	0,107	0,109	0,110	0,111	0,112	0,113	0,114
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,155	0,155	0,156	0,156	0,157	0,157	0,158	0,158	0,159	0,160	0,159	0,016
10% sugar	0,210	0,218	0,225	0,231	0,236	0,241	0,245	0,249	0,251	0,253	0,254	0,023
20% sugar	0,131	0,134	0,137	0,139	0,142	0,144	0,145	0,146	0,147	0,147	0,147	0,012
30% sugar	0,114	0,114	0,114	0,114	0,114	0,114	0,114	0,115	0,117	0,119	0,121	0,004
40% sugar	0,113	0,114	0,115	0,117	0,118	0,120	0,121	0,122	0,124	0,125	0,125	0,019
50% sugar	0,116	0,119	0,121	0,123	0,125	0,127	0,128	0,129	0,129	0,130	0,131	0,024

Table 9: Mean optical density values of strain MO192, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,093	0,092	0,092	0,092	0,091	0,091	0,092	0,092	0,092	0,092	0,093	0,095
10% sugar	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,093	0,093	0,094	0,096
20% sugar	0,094	0,094	0,093	0,093	0,093	0,093	0,093	0,093	0,092	0,092	0,092	0,093
30% sugar	0,094	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
40% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093	0,093	0,093	0,093
50% sugar	0,094	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,101	0,106	0,114	0,125	0,128	0,129	0,128	0,129	0,130	0,130	0,129	0,101
10% sugar	0,103	0,110	0,119	0,131	0,144	0,156	0,167	0,179	0,191	0,202	0,214	0,103
20% sugar	0,094	0,095	0,097	0,099	0,102	0,106	0,117	0,118	0,124	0,129	0,135	0,094
30% sugar	0,093	0,094	0,094	0,095	0,096	0,096	0,097	0,098	0,100	0,101	0,103	0,093
40% sugar	0,093	0,093	0,094	0,094	0,094	0,095	0,096	0,095	0,095	0,095	0,096	0,093
50% sugar	0,093	0,094	0,094	0,094	0,094	0,095	0,096	0,095	0,095	0,095	0,096	0,093
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,129	0,129	0,129	0,129	0,128	0,128	0,128	0,128	0,128	0,127	0,127	0,009
10% sugar	0,226	0,238	0,250	0,261	0,272	0,281	0,290	0,297	0,304	0,310	0,315	0,023
20% sugar	0,141	0,147	0,152	0,157	0,162	0,167	0,172	0,176	0,179	0,182	0,184	0,015
30% sugar	0,104	0,105	0,107	0,108	0,109	0,110	0,111	0,112	0,113	0,113	0,114	0,009
40% sugar	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,095	0,002
50% sugar	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,095	0,002

Table 10: Mean optical density values of strain MO193, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,093	0,093	0,095	0,095	0,095	0,095	0,094	0,095	0,095	0,095	0,095	0,095
10% sugar	0,096	0,096	0,098	0,098	0,097	0,098	0,097	0,097	0,098	0,098	0,099	0,100
20% sugar	0,095	0,095	0,097	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096	0,096
30% sugar	0,096	0,095	0,097	0,097	0,097	0,097	0,096	0,097	0,096	0,097	0,097	0,097
40% sugar	0,096	0,095	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,098	0,098
50% sugar	0,095	0,095	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,095
10% sugar	0,101	0,103	0,107	0,113	0,121	0,130	0,139	0,147	0,161	0,173	0,186	0,200
20% sugar	0,096	0,096	0,096	0,096	0,097	0,097	0,098	0,099	0,100	0,101	0,103	0,105
30% sugar	0,097	0,097	0,097	0,098	0,099	0,100	0,101	0,103	0,104	0,105	0,105	0,105
40% sugar	0,098	0,098	0,098	0,099	0,100	0,101	0,103	0,106	0,108	0,112	0,117	0,122
50% sugar	0,097	0,098	0,098	0,098	0,099	0,100	0,100	0,101	0,102	0,103	0,104	0,105
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,095	0,095	0,095	0,095	0,096	0,096	0,096	0,096	0,096	0,096	0,095	0,001
10% sugar	0,213	0,226	0,237	0,247	0,256	0,265	0,273	0,280	0,287	0,293	0,297	0,035
20% sugar	0,107	0,109	0,111	0,113	0,116	0,119	0,121	0,124	0,127	0,129	0,131	0,017
30% sugar	0,104	0,103	0,103	0,102	0,101	0,101	0,100	0,100	0,100	0,099	0,099	0,001
40% sugar	0,127	0,132	0,135	0,137	0,140	0,142	0,143	0,145	0,146	0,147	0,148	0,010
50% sugar	0,105	0,105	0,105	0,105	0,105	0,104	0,104	0,104	0,105	0,104	0,104	0,004

Table 11: Mean optical density values of strain MO194, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,092	0,093
10% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
20% sugar	0,094	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093	0,093	0,093
30% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093	0,093	0,093
40% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,094	0,093	0,093	0,092	0,092
50% sugar	0,094	0,094	0,094	0,094	0,093	0,094	0,094	0,094	0,094	0,093	0,093	0,092
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,093	0,095	0,097	0,100	0,106	0,113	0,119	0,122	0,125	0,128	0,130	0,132
10% sugar	0,095	0,097	0,100	0,105	0,111	0,120	0,129	0,140	0,151	0,162	0,173	0,184
20% sugar	0,093	0,094	0,094	0,095	0,096	0,099	0,102	0,105	0,111	0,115	0,121	0,127
30% sugar	0,092	0,092	0,093	0,093	0,093	0,093	0,094	0,094	0,095	0,095	0,096	0,097
40% sugar	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092
50% sugar	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,133	0,135	0,137	0,138	0,139	0,140	0,140	0,140	0,140	0,140	0,140	0,017
10% sugar	0,196	0,208	0,220	0,231	0,242	0,252	0,261	0,269	0,276	0,282	0,289	0,064
20% sugar	0,133	0,139	0,144	0,150	0,155	0,160	0,165	0,169	0,173	0,176	0,179	0,028
30% sugar	0,098	0,099	0,100	0,101	0,102	0,103	0,104	0,104	0,105	0,105	0,105	0,008
40% sugar	0,092	0,092	0,092	0,092	0,093	0,093	0,093	0,093	0,093	0,093	0,092	0,001
50% sugar	0,092	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,001

Table 12: Mean optical density values of strain MO229, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,093	0,093	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,096	0,096	0,096
10% sugar	0,093	0,093	0,093	0,093	0,094	0,094	0,095	0,095	0,096	0,097	0,098	0,098
20% sugar	0,093	0,094	0,094	0,094	0,094	0,094	0,095	0,095	0,096	0,096	0,096	0,096
30% sugar	0,093	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,096	0,096	0,096	0,096
40% sugar	0,094	0,095	0,095	0,095	0,095	0,095	0,096	0,096	0,096	0,097	0,097	0,097
50% sugar	0,093	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,096	0,096	0,096	0,097
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,097	0,097	0,097	0,097	0,097	0,097	0,098	0,098	0,098	0,098	0,098	0,098
10% sugar	0,099	0,101	0,104	0,108	0,112	0,119	0,126	0,134	0,142	0,150	0,159	0,169
20% sugar	0,096	0,097	0,097	0,097	0,097	0,098	0,098	0,099	0,099	0,100	0,101	0,102
30% sugar	0,096	0,097	0,097	0,097	0,098	0,098	0,099	0,099	0,101	0,102	0,103	0,105
40% sugar	0,097	0,097	0,098	0,098	0,099	0,099	0,099	0,099	0,100	0,101	0,101	0,102
50% sugar	0,097	0,097	0,097	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,098	0,097	0,098	0,097	0,097	0,097	0,097	0,097	0,096	0,096	0,096	0,004
10% sugar	0,179	0,189	0,198	0,205	0,211	0,216	0,221	0,225	0,229	0,233	0,235	0,031
20% sugar	0,103	0,103	0,105	0,106	0,107	0,108	0,109	0,111	0,112	0,113	0,114	0,012
30% sugar	0,106	0,106	0,106	0,106	0,105	0,105	0,105	0,104	0,104	0,103	0,103	0,003
40% sugar	0,103	0,104	0,105	0,106	0,108	0,109	0,110	0,111	0,112	0,113	0,114	0,005
50% sugar	0,098	0,098	0,099	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,003

Table 13: Mean optical density values of strain MO230, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,094	0,093	0,094	0,094	0,093	0,094	0,094	0,094	0,094	0,094	0,095	0,095
10% sugar	0,094	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,095	0,095	0,096	0,097
20% sugar	0,094	0,093	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
30% sugar	0,094	0,093	0,093	0,093	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093
40% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
50% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,097	0,099	0,103	0,109	0,119	0,134	0,143	0,143	0,142	0,141	0,140	0,140
10% sugar	0,098	0,100	0,103	0,108	0,114	0,121	0,129	0,138	0,146	0,155	0,164	0,173
20% sugar	0,094	0,094	0,094	0,095	0,095	0,096	0,097	0,099	0,101	0,104	0,108	0,112
30% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,094	0,095
40% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,095
50% sugar	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,094	0,093	0,094	0,093	0,093
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,140	0,139	0,139	0,139	0,139	0,138	0,138	0,138	0,138	0,138	0,137	0,011
10% sugar	0,182	0,190	0,199	0,207	0,214	0,220	0,226	0,231	0,234	0,237	0,239	0,014
20% sugar	0,116	0,120	0,124	0,127	0,131	0,134	0,138	0,141	0,143	0,146	0,147	0,012
30% sugar	0,095	0,096	0,096	0,096	0,097	0,098	0,098	0,098	0,099	0,099	0,099	0,003
40% sugar	0,095	0,096	0,096	0,097	0,098	0,099	0,099	0,100	0,101	0,101	0,101	0,003
50% sugar	0,094	0,094	0,094	0,094	0,094	0,095	0,095	0,095	0,095	0,095	0,095	0,001

Table 14: Mean optical density values of strain MO245, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,096	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097
10% sugar	0,096	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097
20% sugar	0,097	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098
30% sugar	0,097	0,097	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,097	0,097	0,097
40% sugar	0,096	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097	0,097
50% sugar	0,097	0,098	0,098	0,098	0,098	0,099	0,098	0,098	0,098	0,098	0,098	0,098
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,097	0,098	0,098	0,099	0,100	0,102	0,105	0,108	0,113	0,121	0,132	0,136
10% sugar	0,098	0,099	0,101	0,104	0,108	0,114	0,122	0,132	0,143	0,153	0,165	0,176
20% sugar	0,098	0,098	0,099	0,100	0,100	0,101	0,103	0,104	0,107	0,110	0,115	0,120
30% sugar	0,097	0,098	0,098	0,098	0,099	0,099	0,100	0,100	0,100	0,100	0,101	0,101
40% sugar	0,097	0,097	0,097	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,099
50% sugar	0,098	0,098	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,098	0,099	0,099
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,136	0,136	0,136	0,136	0,136	0,136	0,136	0,136	0,136	0,136	0,135	0,017
10% sugar	0,186	0,197	0,207	0,217	0,227	0,237	0,246	0,253	0,260	0,266	0,271	0,038
20% sugar	0,126	0,131	0,137	0,142	0,147	0,152	0,156	0,160	0,164	0,166	0,168	0,012
30% sugar	0,102	0,103	0,104	0,105	0,105	0,106	0,106	0,107	0,107	0,107	0,107	0,005
40% sugar	0,099	0,099	0,100	0,100	0,100	0,101	0,101	0,101	0,102	0,101	0,102	0,001
50% sugar	0,099	0,099	0,100	0,100	0,100	0,101	0,101	0,101	0,101	0,101	0,101	0,001

Table 15: Mean optical density values of strain TSN486, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,104	0,096	0,097	0,097	0,097	0,097	0,097	0,094	0,098	0,098	0,095	0,095
10% sugar	0,104	0,096	0,094	0,095	0,095	0,097	0,098	0,097	0,098	0,099	0,098	0,100
20% sugar	0,104	0,097	0,093	0,096	0,096	0,096	0,096	0,096	0,099	0,100	0,097	0,098
30% sugar	0,102	0,097	0,094	0,095	0,095	0,097	0,097	0,096	0,099	0,099	0,098	0,098
40% sugar	0,099	0,096	0,093	0,095	0,095	0,097	0,097	0,097	0,098	0,098	0,096	0,097
50% sugar	0,097	0,097	0,094	0,096	0,096	0,097	0,097	0,097	0,099	0,099	0,097	0,098
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,095	0,095	0,096	0,096	0,096	0,096	0,096	0,097	0,097	0,097	0,097	0,097
10% sugar	0,105	0,110	0,118	0,127	0,136	0,146	0,154	0,163	0,170	0,178	0,186	0,193
20% sugar	0,099	0,102	0,105	0,108	0,113	0,118	0,124	0,130	0,135	0,140	0,144	0,149
30% sugar	0,098	0,098	0,099	0,100	0,101	0,102	0,103	0,104	0,106	0,108	0,110	0,112
40% sugar	0,097	0,097	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,100	0,100	0,101
50% sugar	0,097	0,098	0,098	0,098	0,099	0,099	0,099	0,099	0,099	0,099	0,099	0,099
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,097	0,097	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,098	0,002
10% sugar	0,201	0,207	0,213	0,218	0,222	0,227	0,231	0,234	0,237	0,240	0,243	0,029
20% sugar	0,153	0,157	0,161	0,164	0,168	0,171	0,174	0,177	0,179	0,181	0,182	0,009
30% sugar	0,115	0,117	0,119	0,121	0,122	0,124	0,125	0,126	0,126	0,127	0,127	0,007
40% sugar	0,101	0,102	0,103	0,103	0,104	0,104	0,105	0,105	0,106	0,106	0,106	0,003
50% sugar	0,099	0,100	0,100	0,100	0,101	0,101	0,101	0,101	0,101	0,101	0,100	0,002

Table 16: Mean optical density values of strain TSN529, calculated from 15 replicates

	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h
0% sugar	0,104	0,104	0,104	0,104	0,104	0,104	0,104	0,104	0,104	0,103	0,103	0,103
10% sugar	0,095	0,095	0,095	0,095	0,095	0,095	0,095	0,096	0,096	0,097	0,099	0,102
20% sugar	0,096	0,096	0,096	0,096	0,096	0,095	0,095	0,095	0,095	0,095	0,095	0,096
30% sugar	0,095	0,095	0,095	0,095	0,095	0,095	0,094	0,094	0,094	0,094	0,094	0,093
40% sugar	0,095	0,095	0,095	0,095	0,095	0,095	0,094	0,094	0,094	0,093	0,093	0,093
50% sugar	0,095	0,096	0,096	0,096	0,096	0,095	0,095	0,094	0,094	0,094	0,094	0,094
	26 h	**28 h**	**30 h**	**32 h**	**34 h**	**36 h**	**38 h**	**40 h**	**42 h**	**44 h**	**46 h**	**48 h**
0% sugar	0,102	0,102	0,102	0,102	0,102	0,102	0,101	0,101	0,101	0,101	0,100	0,100
10% sugar	0,108	0,116	0,126	0,135	0,144	0,152	0,159	0,165	0,171	0,177	0,182	0,187
20% sugar	0,096	0,098	0,101	0,105	0,110	0,116	0,121	0,126	0,130	0,134	0,138	0,141
30% sugar	0,094	0,094	0,095	0,096	0,097	0,099	0,102	0,105	0,110	0,114	0,119	0,122
40% sugar	0,093	0,093	0,093	0,093	0,093	0,093	0,093	0,094	0,095	0,096	0,100	0,105
50% sugar	0,093	0,093	0,093	0,093	0,093	0,092	0,092	0,092	0,092	0,092	0,093	0,092
	50 h	**52 h**	**54 h**	**56 h**	**58 h**	**60 h**	**62 h**	**64 h**	**66 h**	**68 h**	**70 h**	**STAB**
0% sugar	0,100	0,100	0,100	0,100	0,100	0,100	0,099	0,099	0,099	0,098	0,098	0,028
10% sugar	0,192	0,197	0,201	0,204	0,208	0,211	0,214	0,216	0,218	0,220	0,221	0,016
20% sugar	0,144	0,147	0,150	0,152	0,155	0,157	0,158	0,160	0,161	0,162	0,163	0,008
30% sugar	0,126	0,128	0,131	0,132	0,134	0,135	0,137	0,138	0,138	0,138	0,139	0,012
40% sugar	0,109	0,113	0,116	0,117	0,119	0,119	0,119	0,119	0,119	0,119	0,118	0,009
50% sugar	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,092	0,091	0,091	0,002

Table 17: R (Vegan) NMDS-script (Figure 18)

```
> data.nmds <- metaMDS(data, distance="euclidean")
Run 0 stress 0.0261442
Run 1 stress 0.03080897
Run 2 stress 0.06566628
Run 3 stress 0.02875691
Run 4 stress 0.0341871
Run 5 stress 0.02691089
Run 6 stress 0.05050096
Run 7 stress 0.0671312
Run 8 stress 0.06822741
Run 9 stress 0.03928384
Run 10 stress 0.03527096
Run 11 stress 0.02625423
... procrustes: rmse 0.001818666  max resid 0.009274497
*** Solution reached
> site.sc <- scores(data.nmds, display="sites")
> site.sc
                  NMDS1         NMDS2
2A-28-C_0   -0.1049011639 -0.1054604107
2A-28-C_10  -0.4137036884 -0.0791846127
2A-28-C_20  -0.1848505150 -0.0415122537
2A-28-C_30  -0.0098404034 -0.0390352659
2A-28-C_40   0.0590214838 -0.0448036497
2A-28-C_50   0.1285307114 -0.0438535013
MO172-C_0    0.1809174108 -0.0140454288
MO172-C_10  -0.4482147227  0.0173619978
MO172-C_20  -0.0654148502 -0.0005144444
MO172-C_30   0.1519068961 -0.0183154050
MO172-C_40   0.1621108025 -0.0221827595
MO172-C_50   0.1650507870 -0.0293231156
MO174-C_0    0.1779354975 -0.0071398830
MO174-C_10  -0.4028115500 -0.0338097371
MO174-C_20  -0.2044394005 -0.0068280504
MO174-C_30   0.1525634630 -0.0119007310
MO174-C_40   0.1339603410 -0.0190746083
MO174-C_50   0.1540538362 -0.0217919752
MO182-C_0    0.1577243763  0.0467669536
MO182-C_10  -0.2303059550  0.0870186663
MO182-C_20  -0.0185247968  0.0439174761
MO182-C_30   0.1375620114  0.0355891176
MO182-C_40   0.1207991897  0.0511801060
MO182-C_50   0.1426507363  0.0336538572
MO184-C_0   -0.1093513294 -0.0921980364
MO184-C_10  -0.3780016800 -0.0226735443
MO184-C_20  -0.0144385147 -0.0069387186
MO184-C_30   0.0748535382 -0.0211359560
MO184-C_40   0.0639206547 -0.0218285058
MO184-C_50   0.0454871169 -0.0278415486
MO190-C_0    0.0156635883  0.0171015959
MO190-C_10  -0.5041037189  0.0146044809
MO190-C_20  -0.0916047103  0.0213699506
MO190-C_30   0.1028640226  0.0098297038
MO190-C_40   0.1476507955  0.0130305443
MO190-C_50   0.1476269315  0.0154830207
MO192-C_0   -0.0104202283  0.1160185402
MO192-C_10  -0.3900878956  0.1475536585
MO192-C_20  -0.0660909704  0.1527980286
MO192-C_30   0.1296651287  0.1478843012
MO192-C_40   0.1641945403  0.1396201773
```

```
MO192-C_50   0.1619148008  0.1224821156
MO193-C_0    0.1478209637  0.0049461556
MO193-C_10  -0.4402846548  0.0384958292
MO193-C_20   0.0718221776  0.0134794240
MO193-C_30   0.1246396820 -0.0120182679
MO193-C_40  -0.0002762108 -0.0206143363
MO193-C_50   0.1153793993 -0.0299805426
MO229-C_0    0.1414846915  0.0091098159
MO229-C_10  -0.2651447641  0.0018751404
MO229-C_20   0.1052468295 -0.0016699000
MO229-C_30   0.1156329721 -0.0081929911
MO229-C_40   0.1040224565 -0.0186873557
MO229-C_50   0.1368575828 -0.0271492314
MO230-C_0   -0.0230838094 -0.0772184606
MO230-C_10  -0.2814766632 -0.0355963345
MO230-C_20   0.0272360207 -0.0436704098
MO230-C_30   0.1461227256 -0.0399376460
MO230-C_40   0.1413907055 -0.0396492137
MO230-C_50   0.1532512590 -0.0402917587
MO245-C_0    0.0073378678 -0.0385740725
MO245-C_10  -0.3382187724 -0.0198157458
MO245-C_20  -0.0350214751 -0.0290791459
MO245-C_30   0.1144188392 -0.0373704947
MO245-C_40   0.1307290542 -0.0394531093
MO245-C_50   0.1301097304 -0.0424299788
TSN486-C_0   0.1408873079 -0.0424209480
TSN486-C_10 -0.3290280968 -0.0638780542
TSN486-C_20 -0.1207144290 -0.0328388388
TSN486-C_30  0.0582471268 -0.0394185003
TSN486-C_40  0.1198869795 -0.0408181552
TSN486-C_50  0.1303238111 -0.0416617078
TSN529-C_0   0.1320135626  0.0817936595
TSN529-C_10 -0.2866340540  0.0548885450
TSN529-C_20 -0.0684589492  0.0425620251
TSN529-C_30  0.0182210683  0.0454561734
TSN529-C_40  0.0822490068  0.0489507928
TSN529-C_50  0.1614874915  0.0190054883
> cols2 <- c("black", "blue", "darkred", "darkgreen", "pink", "yellow")
> sugar <- factor(eco$conc)
> sugar
 [1] C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10
C_20
[16] C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40
C_50
[31] C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10
C_20
[46] C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40
C_50
[61] C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10 C_20 C_30 C_40 C_50 C_0  C_10
C_20
[76] C_30 C_40 C_50
Levels: C_0 C_10 C_20 C_30 C_40 C_50
> cols2[sugar]
 [1] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
 [7] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[13] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[19] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[25] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[31] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[37] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[43] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[49] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[55] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
```

```
[61] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[67] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
[73] "steelblue" "darkred"   "darkgreen" "pink"      "yellow" "orange"
> plot(site.sc, col=cols2[sugar], pch=16)
> ordispider(site.sc, eco$conc, label=TRUE, lty="dotted")
> stressplot(data.nmds)
```

Table 18: Standard deviation values of *C. victoriae* OD-measurements at 70 hours

	2A-28	MO 172	MO 174	MO 182	MO 184	MO 192	MO 193	MO 194	MO 229	MO 230	MO 245	TSN 486	TSN 529
0%	0,007	0,002	0,001	0,001	0,016	0,009	0,001	0,017	0,031	0,011	0,017	0,002	0,028
10%	0,013	0,041	0,017	0,038	0,023	0,023	0,035	0,064	0,012	0,014	0,038	0,029	0,016
20%	0,007	0,020	0,051	0,023	0,012	0,015	0,017	0,028	0,003	0,012	0,012	0,009	0,008
30%	0,005	0,005	0,002	0,003	0,004	0,009	0,001	0,008	0,005	0,003	0,005	0,007	0,012
40%	0,002	0,004	0,003	0,005	0,019	0,002	0,010	0,001	0,003	0,003	0,001	0,003	0,009
50%	0,002	0,002	0,004	0,002	0,024	0,002	0,004	0,001	0,031	0,001	0,001	0,002	0,002

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9. Erklärung

Hiermit erkläre ich, dass ich die heute eingereichte Bachelorarbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt sowie Zitate kenntlich gemacht habe. Bei der vorliegenden Bachelorarbeit handelt es sich um in Wort und Bild völlig übereinstimmende Exemplare.
Weiterhin erkläre ich, dass digitale Abbildungen nur die originalen Daten enthalten und in keinem Fall inhaltsverändernde Bildbearbeitung vorgenommen wurde.

Erstgutachter ist: Prof. Dr. Dominik Begerow

Als Zweitgutachterin schlage ich vor: Jun.-Prof. Dr. Julia Bandow

Bochum, den

_____

(Dominik Schmidt)