The lipid composition of the yeast genus *Saccharomycopsis* Schiönning

by

Olihile Moses Sebolai

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Department of Microbial, Biochemical and Food Biotechnology

Faculty of Natural and Agricultural Sciences
University of the Free State
Bloemfontein
South Africa

Supervisor: Prof. J.L.F. Kock

Co-supervisors: Prof. M.S. Smit

Prof. P.W.J. Van Wyk

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This dissertation is lovingly dedicated to my parents, **Rre le Mme Sebolai.**

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CHAPTER 1

Introduction

1.1 Motivation

In order to effectively explore or map the presence of a particular attribute for biotechnological application, it is advantageous to know if any patterns of distribution regarding this characteristic exist across the different taxa of interest. If this is the case, fruitful bioprospective studies can be launched which will have a good chance of locating the best producers of a specific sought after character. Consequently, an important feature of such a characteristic is that it should be conserved and clearly delimited within defined taxa.

The ideal situation is where such a character follows the same distribution pattern across taxa as other more conserved well-established characteristics i.e. metabolic precursors. The latter can then also be used as indicators showing the direction for further exploration of the character of interest. In fungi, such indicators include amongst others, the presence of different series (ω-3 and ω-6) of polyunsaturated fatty acids (PUFAs), which are conserved on ordinal level (Kock and Botha 1998). This phenotypic characteristic provides clues concerning which taxa are most probably capable of producing high value lipids such as arachidonic acid (AA) and γ-linolenic acid (GLA) for biotechnological exploitation. Both fatty acids are used today in cosmetics and as food supplements. Consequently, such a model (include fatty acid (FA) precursors and their high value products) has been used successfully to expose genera such as *Mucor*

capable of producing GLA in applied biotechnological processes (Kock and Botha 1998).

In this study, the construction of a similar model is attempted but this time to forecast and map the distribution of 3-hydroxy oxylipins. These compounds have been shown to have potent biological activity in yeast and mammalian cells and therefore may have biotechnological value (Kock et al. 1998; Kock et al. 1999; Nigam et al. 1996). Since a member of the yeast genus, *Saccharomycopsis* i.e. *Saccharomycopsis malanga*, has previously been found to over-excrete these oxylipins in crystal form, it was decided to study this genus in more detail. The aim is to construct a map consisting of FA precursors necessary for the production of these oxylipins through incomplete β-oxidation and then determine their ability to forecast the type of oxylipin produced.

Consequently, in this study the fatty acid composition of all members of the genus *Saccharomycopsis* will be determined and taxonomically evaluated (Chapter 2). This will be followed by mapping the distribution of 3-hydroxy oxylipins in this genus and evaluating the FA database for its forecasting ability regarding oxylipin production (Chapter 3).

1.2 *Saccharomycopsis* Schiönning

1.2.1 Development of the classification of *Saccharomycopsis* Schiönning

to start of with, the different classification systems with conserved characteristics, used to delimit *Saccharomycopsis*, will be discussed.

The historical development of the classification of the genus *Saccharomycopsis* is perhaps the most convoluted of the ascomycetous yeast genera. In 1870, Reess originally assigned the currently accepted species of this genus to *Endomyces* Reess, a yeast genus then characterised by the formation of true hyphae (Kurtzman and Smith 1998). These hyphae in turn produced yeast phases through budding (blastoconidia), or through arthroconidia formation. In 1903, Schiönning described the new genus *Saccharomycopsis* and included *S. capsularis* and *S. guttulata,* which was first observed by Remak in 1845 (Van der Walt and Scott 1971). The latter species is currently placed in *Cyniclomyces* Van der Walt and D.B. Scott (Phaff and Miller 1998).

The diagnosis of the genus *Saccharomycopsis* was then as follows: "*Cells formed double walled ascospores, true septate mycelium and budding cells.*"

In 1908 and 1909, Guilliermond performed comparative studies between *Saccharomycopsis capsularis* (Schiönning) and *Endomyces fibuliger* (Lindner) and transferred *Saccharomycopsis capsularis* to the genus *Endomyces* as *Endomyces capsularis* mainly on the basis that the two species differed insufficiently to be placed in

separate genera. He retained *Saccharomycopsis guttulata* under the genus *Saccharomycopsis* (Van der Walt and Scott 1971).

In 1924 Klöcker retained the genus *Saccharomycopsis* Schiönning with *Saccharomycopsis capsularis* as the type and transferred *Endomyces fibuliger* with two other species to this genus. He assigned this genus together with the genus *Endomyces* to the family *Endomycetaceae* (Van der Walt and Scott 1971).

The diagnosis of the family *Endomycetaceae* was given by Schroeter in 1892 (Von Arx 1972) as follows: *"Septate hyphae present, often disarticulating early, vegetative reproduction mostly by arthrospore, or aleuriospores, asci formed laterally from crozier-like cells or mostly after copulation of gametangial hyphae, cylindrical or spherical, one to many-spored, ascospores 1-celled, ellipsoidal or spherical, often surrounded by a sheath, or conglobate, containing refractive bodies."*

In 1931, on the basis of these characteristics, Stelling-Dekker subdivided *Endomyces* and placed the yeast species in *Endomycopsis* Dekker and reserved the genus name *Endomyces* for those species that only form arthroconidia (Van der Walt and Scott 1971). Later, a number of mycelial species such as *Ambrosiozyma monospora, A. platypodis, Pichia bisporis,* and *P. burtonii* were assigned to *Endomycopsis*.

It was later shown by Van der Walt and Scott (1971) that *Endomycopsis* Dekker was illegitimate according to the rules of the Botanic Code and consequently reinstated

Saccharomycopsis Schiönning with the following conserved characteristics: *"…budding cells, true septate mycelium and ascospores with double membranes."* Consequently, they placed certain *Endomycopsis* species within this genus i.e. *Ambrosiozyma monospora*.

Following this, several changes occurred. In 1972, Van der Walt described the genus *Ambrosiozyma* that includes the mycelial species with dolipore-like septa. At this time, Von Arx (1972) described *Hormoascus* for the nitrate assimilating species of *Ambrosiozyma.* In 1985, *Hormoascus* was amended to include both nitrate-assimilating and non-assimilating species (Van der Walt and Von Arx 1985). *Saccharomycopsis synnaedendra* (conserved character: produced no budding cells) was placed under *Botryoascus* and *Endomycopsis (Endomyces) javanensis* (conserved character: forms spheroidal ascospores and buds on a broad base) was placed under *Arthroascus.* This was followed by the transference of *Saccharomycopsis lipolytica* to the genus *Yarrowia* and *S. vini* and *S. crataegensis* to *Endomycopsella* (Van der Walt and Von Arx 1980). In 1984, Von Arx and Yarrow returned *Saccharomycopsis fibuligera* to *Endomyces.* However, the delimitation of the latter genus from *Dipodascus* and *Saccharomycopsis* is controversial since the conserved status of the blastoconidium and arthroconidium formation as well as ascospore morphology and ultrastructure of hyphal septa have been interpreted in many ways (Kurtzman and Smith 1998).

In an effort to restore the nomenclatural stability of the ascomycetous yeasts Kurtzman and Robnett (1995) presented a phylogenetic tree showing that the species at present placed under *Saccharomycopsis* form a distinct clade (Figure 1).

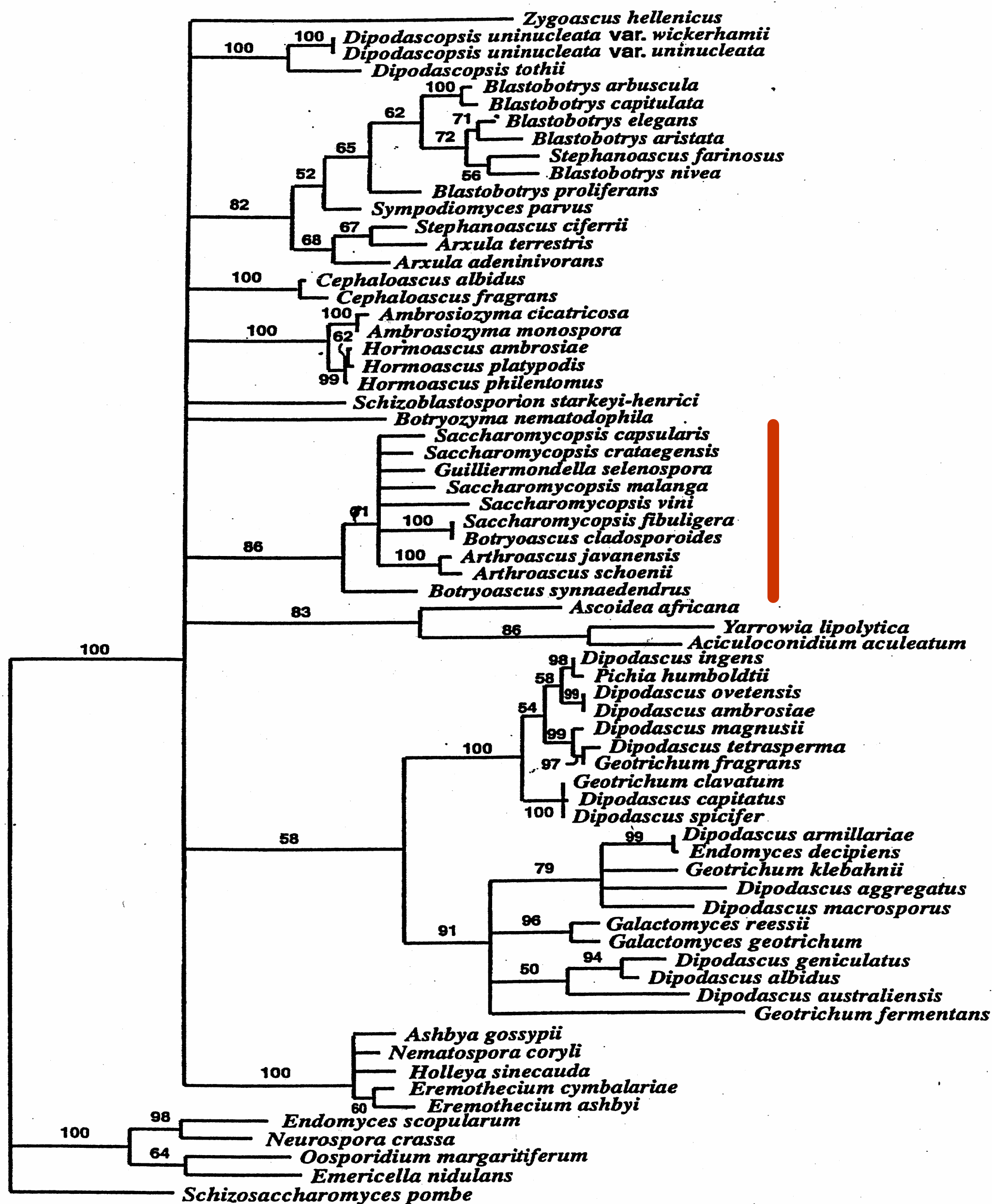


Figure 1: Phylogenetic tree based on the 26S rDNA sequence data analysis showing the mycelial ascomycetous yeasts, yeast-like fungi, and various reference species. The *Saccharomycopsis* species form a distinct clade/monophyletic group that is separate from other mycelial genera (Reproduced from Kurtzman and Robnett 1995).

These taxa are separated from other mycelial genera and species such as *Pichia bispora, P. burtonii, P. scolyti* and *P. wickerhamii* (Kurtzman and Smith 1998). It was further found that species from *Saccharomycopsis* are characterised by species-specific variation in ascospore shape (may be hat-shaped or spheroidal to elongate, with or without equatorial ridges) while hyphal septa may be multiperforated (most species) or containing a single central micropore (*S. javanensis*). On the basis of rDNA nucleotide sequences, ascospore shape and septum ultrastructure are considered not to be conserved on genus level (Kurtzman and Smith 1998).

1.2.2 Species currently accepted (Kurtzman and Smith 1998)

1. *Saccharomycopsis capsularis* Schiönning (1903)*
2. *Saccharomycopsis crataegensis* Kurtzman and Wickerham (1973)*
3. *Saccharomycopsis fermentans* (Lee, Lee, Hsu and Phaff) Kurtzman and Robnett (1995)*
4. *Saccharomycopsis fibuligera* (Lindner) Klöcker, Kurtzman and Robnett (1995)*
5. *Saccharomycopsis javanensis* (Klöcker) Kurtzman and Robnett (1995)*
6. *Saccharomycopsis malanga* (Dwidjoseputro) Kurtzman, Vesonder and Smiley (1974)*
7. *Saccharomycopsis schoenii* (Nadson and Krasil'nikov) Kurtzman and Robnett (1995)*
8. *Saccharomycopsis selenospora* (Nadson and Krasil'nikov) Kurtzman and Robnett (1995)*

9. *Saccharomycopsis synnaedendra* Scott and Van der Walt (1971)*

10. *Saccharomycopsis vini* (Kreger-van Rij) Van der Walt and Scott (1971)*

*References included in Kurtzman and Smith (1998).

1.2.3 Present diagnosis of the genus (Kurtzman and Smith 1998)

"Abundant development of true mycelium, often with blastoconidia. Budding cells are present and may be formed on a broad or narrow base. Arthroconidia are produced by some species. Hyphal septa are usually multiperforate, but may be limited to a single central pore. Asci are usually attached to hyphae and produce 1-4, or rarely 8, ascospores. Ascospores may be hat shaped, reniform with terminal appendages, spheroidal or ellipsoidal, and may have one or more ledges; surfaces may be smooth or roughened. Some species are fermentative. Nitrate is not assimilated. Coenzyme Q-8 is the predominant ubiquinone. Diazonium blue B reaction is negative."

Key to species (Kurtzman and Smith 1998)

1.	a- Galactose assimilated	*S. selenospora*
	b- Galactose not assimilated	2
2 (1).	a- L-Rhamnose assimilated	*S. synnaedendra*
	b- L-Rhamnose not assimilated	3
3 (2).	a- Cellobiose assimilated	4
	b- Cellobiose not assimilated	6
4 (3).	a- Inositol weakly or strongly assimilated	*S. fibuligera*
	b- Inositol not assimilated	5

5 (4).	a- Growth at 37°C	*S. malanga*
	b- Absence of growth at 37°C	*S. capsularis*
6 (3).	a- Glucitol assimilated	7
	b- Glucitol not assimilated	8
7 (6).	a- D-Gluconate assimilated	*S. crataegensis*
	b- D-Gluconate not assimilated	*S. vini*
8 (6).	a- Glucose fermented	*S. fermentans*
	b- Glucose not fermented	9
9 (8).	a- N-Acetyl-D-glucosamine assimilated	*S. javanensis*
	b- N-Acetyl-D-glucosamine not assimilated	*S. schoenii*

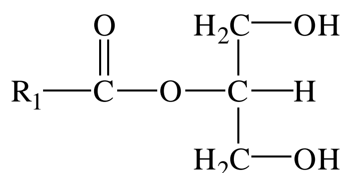
1.3 Lipids in yeasts

1.3.1 Definition

Lipids are generally defined on the basis of their solubility in solvents. They are more readily soluble in organic solvents such as chloroform, hydrocarbons, ethers and esters than in water (Ratledge and Wilkinson 1988). They are divided into two groups (1) those lipids that contain the isoprene unit i.e. terpenoid lipids and, (2) the FA based lipids. In fungi, FAs are rarely found in their free form and are found either as hydroxy fatty acids (oxylipins) or as a rule esterified to a glycerol backbone to form among others phospho- (PL), glyco - (GL), sphingolipids (SL) and triacylglycerols (TAGs) (Figure 2). Therefore,

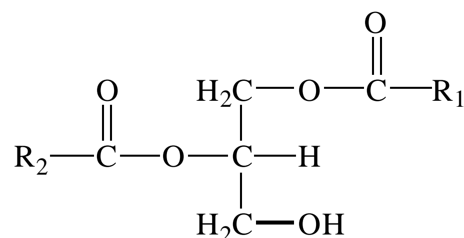
because part of the aim of this study is to map the lipid composition of this genus and to evaluate its taxonomic value, these FA based lipids merit further discussion.

Monoacylglycerol



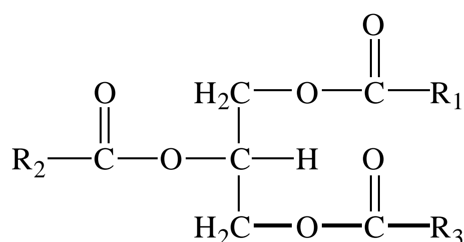
2-Acyl-*sn*-glycerol

Diacylglycerol



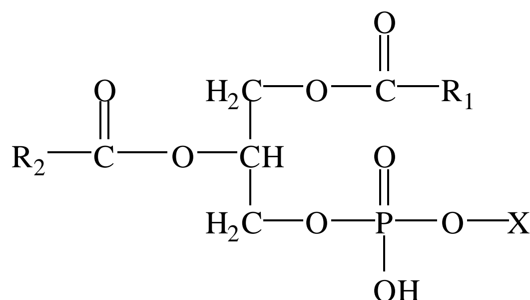
1,2-Diacyl-*sn*-glycerol

Triacylglycerol



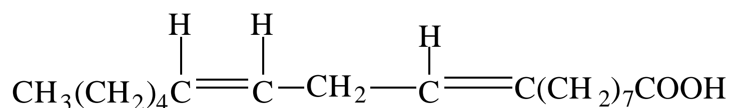
1,2,3-Triacyl-*sn*-glycerol

Phospholipid



Phosphatidic acid

Free fatty acid



Linoleic acid (C18:2)

Figure 2: Fatty acid (FA) derivatives. R_1 CO-, R_2 CO-, R_3 CO- represent fatty acyl groups. X = different ligands can be esterified at this point i.e. hydrogen, choline, serine, etc. (Ratledge and Wilkinson 1988).

The FAs of fungi, particularly yeasts, comprise mainly of long chains of even numbered carbon atoms (i.e. C16 and C18) and include palmitic- (16:0), palmitoleic- (16:1), stearic- (18:0), oleic- (18:1), linoleic- (18:2) and linolenic acids (18:3) (Kock and Botha 1998) with, the ω-3 and ω-6 series of PUFAs being the predominant FA-families. Both, the ω-3 and ω-6 series of PUFAs are derived from 18:2 through participation of different desaturase and elongase enzymes (Van der Westhuizen 1993) (Figure 3).

It has been reported that the FA composition could be affected by cultivation procedures (Deinema 1961). Consequently, a standardised technique was developed which include growth conditions, nature of the medium, extraction and analysis of FAs (Kock et al. 1985; Viljoen et al. 1986) in order to produce reproducible FA results that could be compared for identification purposes. According to literature the following factors *viz.* growth rate, culture age, oxygen availability, temperature, pH and composition of the growth medium should be taken into account if comparative studies on the cellular FA composition of microorganisms are to be conducted, since these factors can alter their FA composition (Erwin 1973; Rattray 1988).

1.3.2 Fatty acid distribution in fungi

When a comparison is made between the distribution of the ω-3 and ω-6 series of PUFAs and the evolutionary development of fungi as proposed by Kendrick (1992), informative patterns are observed (Figure 4). The protoctistan fungi i.e. Oomycota, Chytridiomycota and Hyphochytridiomycota, which are considered to be “ancestral” to the true fungi, are generally characterized by flagellated zoospores, sporangia and the

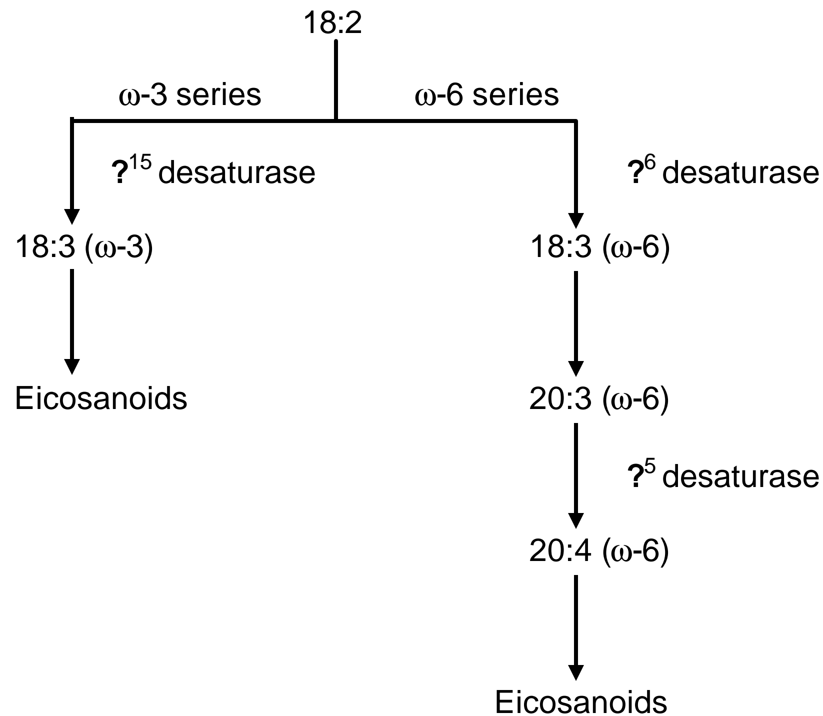


Figure 3: The production of the ω-3 and ω-6 series of polyunsaturated fatty acid (PUFAs). Linoleic acid (18:2) serves as the precursor for the production of both the ω-3 and ω-6 series of the PUFAs through the actions of elongase and desaturase enzymes. 18:2 = linoleic acid, 18:3 (ω-3) = α-linolenic acid, 18:3 (ω-6) = γ-linolenic acid, 20:3 (ω-6) = dihomo-γ-linolenic acid and, 20:4 (ω-6) = arachidonic acid (Van der Westhuizen 1993).

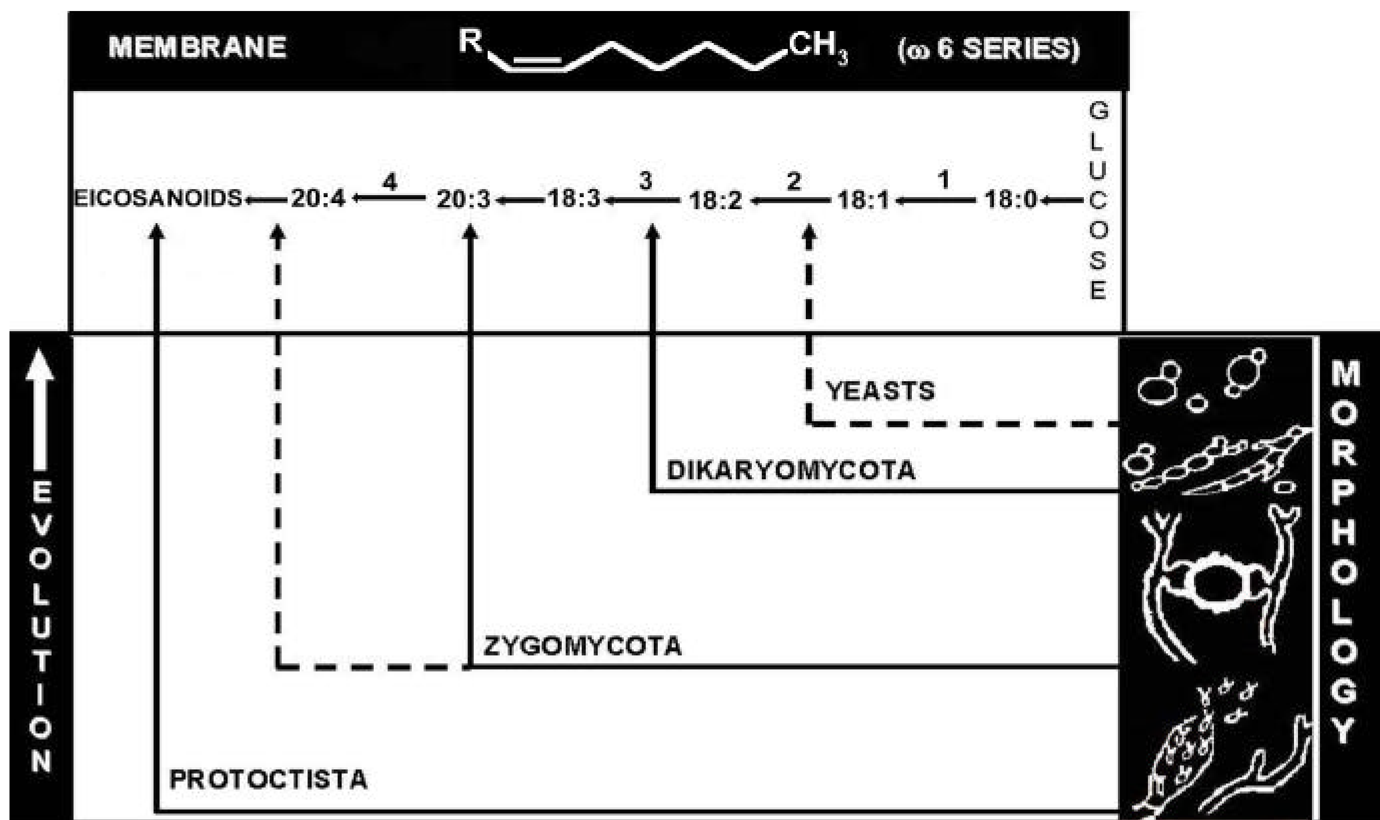


Figure 4: The distribution of the ω-3 and ω-6 series of polyunsaturated fatty acids (PUFAs) in fungi. This coordinated scheme depicts the evolutionary development of fungi based on morphology and PUFA production in membranes - indicating a reduction in FA characteristics along with development. R = $(CH_2)_nCOOH$, 1 = Δ^9 desaturase; 2 = Δ^{12} desaturase; 3 = Δ^6 desaturase; 4 = Δ^5 desaturase, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = γ-linolenic acid, 20:3 = dihomo-γ-linolenic acid and, 20:4 = arachidonic acid (Van der Westhuizen 1993).

presence of the ω-6 series of PUFAs comprising of a hydrocarbon chain length of eighteen (C18) to twenty (C20) carbons (Kock and Botha 1998).

These water-living fungi most probably gave rise to the non-motile terrestrial fungi *viz.* zygomycotan and dikaryomycotan fungi. The zygomycotan fungi seem to be more closely related to the protoctistan fungi in that they possess sporangia as well as coenocytic mycelia, while the dikaryomycotan fungi have septate mycelia and do not produce sporangia. Concerning the FA distribution of both the zygomycotan and dikaryomycotan fungi the following patterns are observed (Kock and Botha 1998):

(i) Zygomycotan fungi produce the ω-6 series of PUFAs, although most representatives only have C18 and not C20 PUFAs.

(ii) Dikaryomycotan fungi and their anamorphs do not produce the ω-6 series of PUFAs. Some are characterized by the presence of 18:3 (ω-3) and others can only produce FAs up to 18:2 (ω-6).

Consequently, through years of fungal evolution, i.e. from the protoctistan to the recently evolved fungi, we notice the emergence of the ω-3 series of PUFAs and the reduction in the FAs chain length. The FA distribution and the evolutionary development of fungi are in agreement with the inferred 18S rRNA sequence variation (Wilmotte et al. 1993). The distribution of PUFAs and 18S rRNA sequence variation are considered crucial evolutionary chronometers and therefore highly conserved characters. This is

probably ascribed to the fact that both these characteristics are important in ensuring the survival of the cell - PUFAs for cell membrane integrity and function and 18S rRNA for protein synthesis (Van der Westhuizen 1993).

1.3.3 Fatty acid profile and yeast taxonomy

Over the years, morphological, biochemical and physiological characteristics have been used to identify yeasts (Barnett et al. 1990; Kreger-van Rij 1984). These systems are however laborious and at times give ambiguous results. In an effort to address this, several chemotaxonomic techniques were developed i.e. classification of isoprenoid quinones in the electron transport chain, electrophoretic enzyme patterns, proton magnetic resonance spectra of cell wall mannans, genome comparison, DNA finger printing as well as lipid profiling which has shown much promise in yeast identification (Kock and Botha 1998). According to literature the latter phenotypic characteristic is not only conserved on ordinal level but is also capable of discriminating between species within a genus. This character also has the potential to be used as a bioprospecting tool in order to locate fungi that are capable of producing high value lipids with biotechnological application i.e. GLA and AA (Kock and Botha 1998).

1.4 Oxylipins

1.4.1 Definition

Oxylipins can be defined as saturated or unsaturated hydroxy fatty acids (Bhatt et al. 1998) or oxygenated fatty acid derivatives (Venter et al. 1997). These are normal fatty acids that contain a hydroxyl group. These compounds include (1) eicosanoids, which have potent biological activities and play an important role in cellular function and (2) hydroxy fatty acids that are produced by either the lipoxygenase, dioxygenase or cytochrome P-450 mediated pathways that are also present in fungi (Van Dyk et al. 1994).

Here, emphasis will be placed on 3-hydroxy fatty acids (oxylipins) as this family of oxylipins has been reported to be present in the genus under investigation. It has been reported (Venter et al. 1997) that these oxylipins are most probably produced from incomplete β-oxidation of FAs.

1.4.2 Distribution and possible function of 3-hydroxy oxylipins in fungi

In 1964, Tulloch and Spencer reported the presence of 3-D hydroxy palmitic acid (16:0) and stearic acid (18:0) as part of the extracellular glycolipids of strains of *Rhodotorula glutinis* and *R. graminis*. Stodola and co-workers (1967) also illustrated the presence of 3-hydroxy oxylipins in yeasts. In 1968, Vesonder and co-workers reported the formation of the extracellular 3-D hydroxy palmitic acid (16:0) by yeast later identified as *Saccharomycopsis malanga* (Kurtzman et al. 1974).

In 1991, Van Dyk and co-workers demonstrated that 3-hydroxy unsaturated fatty acids are produced during the sexual stage of the yeast *Dipodascopsis uninucleata*. They also reported that this yeast is capable of transforming exogenously fed AA to 3-hydroxy 5,8,11,14- eicosatetraenoic acid (3-HETE). This biotransformation process of AA to 3-HETE was also found in *D. tóthii* and *Babjevia anomala* (Kock et al. 1991; Van Dyk et al. 1991; Coetzee et al. 1992).

The distribution of 3-hydroxy fatty acids is well known in fungi, but their biological activities are still to be elucidated further. It was illustrated by means of immunofluorescence microscopy (Kock et al. 1998) that 3-hydroxy oxylipins are produced during the sexual stage of the yeast *D. uninucleata*, and consequently perform a regulatory function in its sexual reproductive stage. In 1999, Kock and co-workers found that these oxylipins play an important role in the release and aggregation of sexual spores of this yeast. Other oxylipins called the precocious sexual inducers or psi factors are also known to have a regulatory role during the sexual reproductive stage of fungi (Kock et al. 1998).

The biological activity of the oxylipin 3-HETE, produced by *D. uninucleata*, was also studied in humans. The results showed that 3-HETE affects the signal transduction processes in human neutrophils and tumor cells in many ways (Nigam et al. 1996). 3-Hydroxy 5,8,11,14- eicosatetraenoic acid at concentrations of 10-100 nM, initiated the production of unesterified AA and platelet activating factor formation in FMLP-challenged human neutrophils in a time and concentration-dependant manner.

Following bioprospecting studies, 3-hydroxy oxylipins were found to be closely associated with the surface structures of the aggregating sexual spores and asci of the representative members of the yeast family Lipomycetaceae (Smith et al. 2000a,b) as well with the surfaces of the aggregating vegetative cells of *Saccharomyces cerevisiae* (Kock et al. 2000). Interestingly, it was also uncovered that these oxylipins are associated with the zygomycotan fungi. In 1998, Pohl and co-workers found that *Mucor genevensis* was capable of biotransforming exogenous AA to 3-hydroxy-5,8,-tetradecaenoic acid (3-hydroxy 14:2) via 18:2. Further bioprospecting studies utilising immunofluorescence microscopy, showed that these oxylipins are specifically associated with the columella of some mucoralean fungi (Strauss et al. 2000). The structures of these compounds still await further clarification.

Literature suggests that these oxylipins may act as adhesives in the fungal domain. This possible function is attributed to their entropic-based hydrophobic nature and ability to form hydrogen bonds in order to effect aggregation or adhesion of cells in fungi (Kock et al. 2003). It is also proposed that these oxylipins can act as a prehistoric lubricant assisting in the liberation of ascospores from asci (Kock et al. 2004).

1.5 Purpose of this study

The purpose of this study, considering the previous discussions, became the following:

1. To map the lipid composition of species in the yeast genus *Saccharomycopsis* Schiönning and evaluate its taxonomic value (Chapter 2).

2. To map the distribution of 3-hydroxy oxylipins in representatives of the yeast genus *Saccharomycopsis* Schiönning (Chapter 3).

3. To determine if FA composition can be used to forecast the presence of certain 3-hydroxy oxylipins in this yeast genus, especially since these oxylipins are produced from incomplete β-oxidation of FAs.

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CHAPTER 2

Lipid composition of the yeast genus *Saccharomycopsis* Schiönning and its taxonomic value

2.1 Abstract

In this study, the distribution of cellular long chain fatty acids in representatives of *Saccharomycopsis* when grown under standardised conditions was mapped and its taxonomic status and level of conservation determined. By using column chromatography to analyse the different lipid fractions and gas chromatography to determine the respective fatty acid methyl esters, it was possible to identify nine of the 10 species i.e. *Saccharomycopsis capsularis, S. crataegensis, S. fibuligera, S. javanensis, S. malanga, S. schoenii, S. selenospora, S. synnaedendra* and *S vini* with the exception of *S. fermentans. Saccharomycopsis crataegensis* was unique since it produced by far the highest percentage neutral lipids (52.4% w/w) while *S. schoenii* produced the highest percentage phospholipids (35.9% w/w). All strains produced palmitic- (16:0), stearic- (18:0), oleic- (18:1) and linoleic acid (18:2) in all lipid fractions analysed. The major fatty acids (FAs) produced were 18:1 and 18:2, while palmitoleic- (16:1) and α-linolenic acid [18:3 (ω-3)] varied between species. *Saccharomycopsis capsularis* produced the highest percentage 18:2 in the neutral lipid fraction while *S. crataegensis, S. malanga* and *S. selenospora* produced the highest percentages of 18:1, 18:0 and, 18:3 (ω-3) respectively, in the neutral lipids. *Saccharomycopsis vini* produced the lowest percentage 16:0 in this fraction. *Saccharomycopsis fibuligera* and *S. schoenii* produced the highest percentages of 16:0 and 18:2 respectively in the

glycolipid fraction. *Saccharomycopsis javanensis* and *S. synnaedendra* produced the highest percentages of 18:1 and 16:1 respectively in the phospholipid fraction.

2.2 Introduction

The historical development of the classification of the genus *Saccharomycopsis* is the most convoluted among the ascomycetous yeasts (Kurtzman and Smith 1998). In 1903, Schiönning initially described the genus *Saccharomycopsis* with *S. capsularis* as the type species on the basis of the cardinal diagnosis of double walled ascospores, true mycelia and budding cells (Van der Walt and Scott 1971). Over the years, taxonomists used different criteria in order to classify this genus. This led to certain species within this genus being assigned to other genera. For instance, in 1931 Stelling-Dekker placed certain species of this genus in *Endomycopsis* and *Endomyces* on the basis of their mode of reproduction (Van der Walt and Scott 1971). At present, this genus has 10 assigned species (Kurtzman and Smith 1998).

In fungi, lipids are reported to be conserved at division and also at species level (i.e. strains of a species will have the same FA profile). As a result of this, cellular long-chain FAs have been used successfully over the years in order to classify yeasts (Lösel 1988; Kock and Botha 1993, 1998). In 1991 Augustyn and co-workers were able to differentiate the *Saccharomyces sensu lato* from the *sensu stricto* groups by determining the relative percentages of 18:2 and 18:3 produced by these two groups. The *Saccharomyces sensu lato* was found to produce higher relative percentages of 18:2 and 18:3 as compared to the *sensu stricto* group. When the yeast genus *Nadsonia* was

revised, the FA composition was used as one of the criteria to differentiate between *N. commutata* and *N. fulvescens,* where the latter was found to produce lower relative percentages of 18:2 and 18:3 than *N. commutata* (Golubev et al. 1989).

Furthermore, in 1988, Kock and co-workers found a correlation between the FA composition and broad evolutionary affinities such as ascospore morphology in the genus *Kluyveromyces.* Consequently, this allowed *Kluyveromyces* to be divided into the "primitive" (i.e. produce higher percentages of 18:2 and 18:3 and are interfertile) and the more "evolved" taxa, which are characterised by low percentages of 18:2 and 18:3 and have lost the ability to cross-fertilise (i.e. genetically isolated). It was also possible to distinguish between *Schizosaccharomyces pombe* and *S. japonicus* on the basis of polyunsaturated fatty acid (PUFA) profiles (Kock and Van der Walt 1986). On the basis of these and other results (ascospore morphology and the presence of co-enzyme Q-10) the latter species was transferred to the genus *Hasegawaea* i.e. produce higher percentages of 18:2 in the total lipid (TL) fraction (Yamada and Banno 1987).

The aim of the present study is to map the lipid composition of the yeast genus *Saccharomycopsis* using gas chromatography in order to evaluate the taxonomic value and conserved status of these FA based lipids.

2.3 Materials and methods

Strains studied

The strains of the 10 currently recognised species of the yeast genus *Saccharomycopsis* (Kurtzman and Smith 1998) were used in this study (Table 1). These are held at the University of the Free State (UOFS), Bloemfontein, South Africa.

Cultivation and harvesting of cells

These strains were cultivated on YM (yeast-malt) agar medium (Wickerham 1951) for two days. Following this, a loopful of cells was inoculated into 500 ml flasks containing 40 ml of standardised YNB (Difco Laboratories) broth medium (nitrogen base 6.7 g l^{-1}, glucose 40 g l^{-1}). The flasks were incubated on a rotary shaker (150 rpm) at 25°C until late exponential phase. Subsequently, appropriate volumes were transferred to 1-litre flasks containing 200 ml of the same YNB broth medium in order to obtain an absorbance reading of 10 Klett units. These flasks were incubated until the stationary phase was reached under set conditions as above (Tredoux et al. 1987). Cells were then harvested at 10 000 rpm for 20 min, washed twice with distilled water (dH_2O), frozen at –70°C and finally freeze dried.

Lipid extraction

This was performed as described by Folch et al. (1957). Here, cells were suspended overnight in chloroform and methanol (2:1, v/v) in order to extract lipids. The extracts

were then washed twice with dH_2O (i.e. dissolve the organic matter), with the eventual evaporation of the organic phase under a stream of nitrogen. Following this, lipids were transferred to pre-weighed vials using diethyl ether. These were dried in a vacuum oven set at 50°C over P_2O_5 before they were finally weighed.

Fractionation of extracted lipids

Extracted lipids were reconstituted in chloroform and then subjected to an activated silicic acid column (heated overnight at 110°C). Elution of the neutral- (NL), glyco- (GL) and phospholipid (PL) fractions was obtained by adding organic solvents (i.e. trichloroethane, acetate and methanol respectively) of different polarities. Diethyl ether was then used to transfer these fractions to pre-weighed vials. Weight determination was performed as described above for the lipid extraction.

Fatty acid analysis

This was performed as described (Butte 1983). Here, the FA methyl esters were obtained after the extracted lipids were transesterified using trimethyl sulphonium hydroxide (TMSOH). The FA methyl ester samples were injected (1 µl) into a Hewlett Parkard 5890 gas chromatograph (Illinois, USA). This instrument is equipped with a Supelcowax 10 polar/capillary column (30 m x 0.35 mm). Nitrogen was used as carrier gas, set at a flow rate of 4 ml min^{-1}. The temperature was programmed to rise by 3°C min^{-1} from an initial column temperature set at 145°C to a final temperature of 240°C. The inlet temperature was set at 180°C. The FA peaks were detected using a flame

ionisation detector (FID) set at 300°C. Peaks were identified by reference to suitable authentic standards.

Chemicals used

All organic chemicals and solvents used were of highest purity grade and obtained from major reputable retailers. Silicic acid (100 mesh) was from Aldrich. NL and PL standards, as well as the FA standards were obtained from Sigma.

2.4 Results and discussion

According to this study, the percentage TL content of the 10 species (Table 2) analysed ranged from 1.7% (w/w) to 4.9% (w/w) in *S. crataegensis* and *S. fibuligera* respectively. Due to this narrow range, this phenotypic characteristic seems to have limited taxonomic value.

The GL fraction was the major lipid type in all species tested (when in stationary growth phase) with the exception of *S. crataegensis* in which the NL fraction was the main fraction. It is reported that during stationary growth phase, as a rule, NLs are the major lipid type due to the depletion of nitrogen sources, which in turn prompts NL accumulation (Van Rensburg 1994).

Saccharomycopsis crataegensis was found to be unique since it contained the highest percentage NLs i.e. 52.4% (w/w) while *S. javanensis* could only produce 2.8% (w/w). Furthermore, *S. javanensis* produced up to 82.1% (w/w) GLs while *S.*

crataegensis only produced 28% (w/w) of this lipid type. *Saccharomycopsis schoenii* produced 35.9% (w/w) PLs while *S. javanensis* could only produce 15.1% (w/w) (Table 2).

The strains studied produced mainly 16:0, 18:0, 18:1 and 18:2 in all lipid fractions analysed. The major FAs produced were 18:1 and 18:2, while 16:1 and 18:3 (ω-3) varied between species (Table 3).

The relative percentages obtained for the long chain PUFAs present in the NL fraction analysed, allowed for five species to be identified. *Saccharomycopsis capsularis,* was found to produce the highest relative percentage 18:2 (48.5% w/w) compared to the rest of the species. *Saccharomycopsis crataegensis* produced a high relative percentage 18:1 (57.9% w/w), while *S. malanga* produced the highest relative percentage 18:0 (31.6% w/w). *Saccharomycopsis selenospora* was characterised by a high relative percentage 18:3 (ω-3) (22.3% w/w), and *S. vini* was characterised by a low relative percentage 16:0 of 4.8% w/w (Table 3).

When the GL fraction was analysed, two species could be identified i.e. *S. fibuligera* and *S. schoenii*, with both species producing the highest relative percentage 16:0 (24.9% w/w) and 18:2 (59.3% w/w) respectively, as compared to the rest of the species. Again *S. selenospora* produced by far the highest percentage 18:3 (ω-3) (28.0% w/w) (Table 4).

The unsaturated FA profiles of the PL fractions allowed for the identification of *S. javanensis* which produced the highest relative percentage 18:1 (47.9% w/w) and *S. synnaedendra* which produced the highest relative percentage 16:1 i.e. 6.3% w/w (Table 5).

Based on the findings of this study, we were able to identify the following *Saccharomycopsis* species namely, *S. capsularis* (18:2 in NLs), *S. crataegensis* (18:1 in NLs), *S. fibuligera* (16:0 in GLs), *S. javanensis* (18:1 in PLs), *S. malanga* (18:0 in NLs), *S. schoenii* (18:2 in GLs), *S. selenospora* [18:3 (ω-3) in NLs], *S. synnaedendra* (16:1 in PLs), and *S. vini* (16:0 in NLs). However, it was not possible to distinguish *S. fermentans* from the rest of the species.

Identification of yeasts using FA profiles provides an alternative method to be used in certain yeast taxa that is rapid compared to the more laborious method based on physiological and morphological characteristics (which are sometimes less accurate).

2.5 Conclusions

On the basis of these results the following conclusions can be drawn:

1. This genus showed a unique lipid composition whereby with the exception of *S. crataegensis,* the GL was the predominant lipid fraction during the stationary phase.

2. We were able to identify nine of the 10 species of this genus through determination of the relative FA percentages produced by the respective species. However, more strains of these species still need to be evaluated before any concrete conclusions can be drawn.

3. Fatty acid profiling proved to be a reliable and rapid identification method as compared to other identification methods. The relationship between this phenotypic character and subsequent 3-hydroxy oxylipin production should now be determined.

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Table 1: *Saccharomycopsis* species used in this study.

Strain studied	Strain number
Saccharomycopsis capsularis	UOFS Y-0447 T
S. crataegensis	CBS 6447
S. fermentans	CBS 7830
S. fibuligera	CBS 329.83
S. javanensis	CBS 2555
S. malanga	CBS 6267 T
S. schoenii	CBS 7223
S. selenospora	CBS 2562 T
S. synnaedendra	CBS 6161
S. vini	CBS 4110

CBS = Centraalbureau voor Schimmelcultures, Delft, The Netherlands and UOFS = University of the Free State, Bloemfontein, South Africa. T = Type strain; Y = Yeast.

Table 2: Total lipid content and the percentage lipid fractions of the 10 *Saccharomycopsis* species when cultivated in liquid glucose-YNB medium.

Species	TL % (w/w)	NL % (w/w)	GL % (w/w)	PL %(w/w)
S. capsularis	2.4	16.1	56.5	27.3
S. crataegensis	1.7	52.4	28.0	19.0
S. fermentans	3.1	9.3	70.3	20.4
S. fibuligera	4.9	5.4	71.2	23.4
S. javanensis	4.3	2.8	82.1	15.1
S. malanga	3.0	10.8	68.1	21.1
S. schoenii	3.4	4.8	58.3	35.9
S. selenospora	3.9	4.7	75.0	20.3
S. synnaedendra	2.9	14.4	70.3	15.3
S. vini	3.2	7.9	74.4	17.6

All experiments were performed in triplicate. Standard error < 5 %.

Table 3: Relative fatty acid percentages obtained in the neutral – (NL) lipid fraction of the 10 *Saccharomycopsis* species when cultivated in liquid glucose-YNB medium.

	NL % (w/w)					
Species	16:0	16:1	18:0	18:1	18:2	18:3 (ω-3)
S. capsularis	18.4	t	11.3	14.7	48.5	4.8
S. crataegensis	8.5	9.6	2.0	57.9	22.0	t
S. fermentans	16.3	4.4	17.9	27.8	29.2	4.3
S. fibuligera	15.6	t	10.1	35.6	38.6	t
S. javanensis	18.2	t	17.1	42.5	19.6	2.7
S. malanga	18.9	t	31.6	19.3	30.3	t
S. schoenii	15.4	t	19.5	36.1	28.9	t
S. selenospora	17.8	2.6	13.5	22.9	20.8	22.3
S. synnaedendra	12.1	3.2	3.4	57.1	24.1	t
S. vini	4.8	2.4	7.3	43.3	42.2	t

All experiments were performed in triplicate. Standard error < 9 %; t = values < 0.5 %. 16:0 = palmitic acid, 16:1 = palmitoleic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α – linolenic acid.

Table 4: Relative fatty acid percentages obtained in the glyco – (GL) lipid fraction of the 10 *Saccharomycopsis* species when cultivated in liquid glucose-YNB medium.

	GL % (w/w)					
Species	16:0	16:1	18:0	18:1	18:2	18:3 (ω-3)
S. capsularis	21.0	2.5	7.9	14.3	54.3	t
S. crataegensis	6.9	12.9	2.0	54.1	24.7	t
S. fermentans	14.8	8.2	4.6	37.5	35.0	t
S. fibuligera	24.9	3.0	5.8	30.8	35.4	t
S. javanensis	23.5	2.5	7.1	40.7	26.1	t
S. malanga	22.9	t	11.8	19.4	44.0	t
S. schoenii	9.5	3.2	1.4	25.8	59.3	t
S. selenospora	14.6	4.5	2.9	23.1	26.9	28.0
S. synnaedendra	14.7	5.5	3.9	46.7	28.6	t
S. vini	16.0	8.8	4.5	33.7	36.9	t

All experiments were performed in triplicate. Standard error < 3 %; t = values < 0.5 %. 16:0 = palmitic acid, 16:1 = palmitoleic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α– linolenic acid.

Table 5: Relative fatty acid percentages obtained in the phospho – (PL) lipid fraction of the 10 *Saccharomycopsis* species when cultivated in liquid glucose-YNB medium.

	PL % (w/w)					
Species	16:0	16:1	18:0	18:1	18:2	18:3 (ω-3)
S. capsularis	27.2	2.5	12.7	20.4	37.2	t
S. crataegensis	13.5	4.1	2.5	36.1	43.7	t
S. fermentans	20.4	3.5	5.2	32.1	38.9	t
S. fibuligera	21.9	2.0	3.1	35.1	35.8	2.2
S. javanensis	23.7	1.7	1.3	47.9	25.4	t
S. malanga	25.2	t	17.7	20.7	36.3	t
S. schoenii	16.7	2.0	1.3	31.8	47.9	t
S. selenospora	28.5	3.0	3.0	32.9	30.3	2.2
S. synnaedendra	18.4	6.3	8.8	36.5	29.9	t
S. vini	19.6	3.8	3.5	30.8	42.3	t

All experiments were performed in triplicate. Standard error < 5 %; t = values < 0.5 %. 16:0 = palmitic acid, 16:1 = palmitoleic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, 18:3 = α – linolenic acid.

Chapter 3

Bioprospecting for novel hydroxy oxylipins in the yeast genus *Saccharomycopsis* Schiönning

This chapter consists of different sections, each in publication form according to the style requested by the journal of submission.

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Section 3.1

Bioprospecting for novel hydroxyoxylipins in fungi: presence of 3-hydroxy palmitic acid in *Saccharomycopsis malanga*

The candidate performed preliminary studies on parts of section 3.1 during his B.Sc. Honours in 2000. After additional work in 2001, parts of this section have been published by him (Antonie van Leeuwenhoek **80:** 311-315, 2001) and also included with

permission in this M.Sc. study (section 3.1). This is the independent work of the candidate.

Authors: Sebolai et al.

3.1.1 Abstract

Electron microscopy studies indicated that the major oxylipin 3-hydroxy palmitic acid (16:0) was associated with aggregating vegetative cells and formed a web-like structure around these cells. Cross sections through this structure showed a hydrophilic outer layer and a more hydrophobic inner layer suggesting that the web-like structure is in fact tube-like micelles. This information sheds more light on the role of these hydroxyoxylipins in fungi.

Key words: Micelles, Hydroxyoxylipins, 3-Hydroxy 16:0, *Saccharomycopsis malanga*

3.1.2 Introduction

It has been reported that yeasts, including *Saccharomycopsis malanga*, are capable of producing various 3-hydroxyoxylipins (Vesonder et al. 1968; Kurtzman et al. 1974; Van Dyk et al. 1991; Venter et al. 1997). Further bioprospecting studies showed that these compounds are mainly associated with the sexual stages (i.e., closely grouped/aggregating ascospores and asci) of members of the yeast family Lipomycetaceae (Kock et al. 1999; Smith et al. 2000a,b) and are present on the surface of aggregating cells of *Saccharomyces cerevisiae* (Kock et al. 2000).

The aim of this study was to further investigate the structure and function of these oxylipins in the yeast *Saccharomycopsis malanga* in order to elucidate the role of these compounds in yeasts.

3.1.3 Materials and methods

Isolate used

Saccharomycopsis malanga (UOFS Y-0666), which is held as a culture at the University of the Orange Free State in South Africa, was used in this study.

Cultivation

The yeast was cultivated on yeast-malt (YM) agar medium at 25°C for 7 days.

Electron microscopy

Material for electron microscopy was chemically fixed with 1.0 M (pH 7) sodium phosphate-buffered gluterdialdehyde (3%) for 3 h and then for 1.5 h in similarly buffered osmium tetroxide (Van Wyk & Wingfield 1991). The material was dehydrated in a graded acetone series. The acetone-dehydrated material for scanning electron microscopy (SEM) was critical-point dried, mounted and coated with gold. This preparation was then examined using a Jeol WINSEM (JSM 6400) scanning electron microscope.

Transmission electron microscopy (TEM) material was embedded in epoxy resin and then polymerized at 70°C for 8 h (Spurr 1969). An LKB III Ultratome was used to cut 60-nm sections with glass knives. Uranyl acetate was used to stain sections for 10 min, followed by lead citrate (Reynolds 1963) for 10 min and the preparation viewed with a Philips EM 100 transmission electron microscope.

Hydroxyoxylipin analysis

Biomass of *S. malanga* [approx. 5.0 g, w/w], obtained from the solid agar medium, was suspended in 200 ml distilled H_2O after which the pH was lowered to 3.5 by the addition of 3% (v/v) formic acid. Subsequently, the oxylipins were extracted by using 2 X 200 ml of ethyl acetate followed by evaporation according to the method of Nigam (1987). Following this, the methyl-trimethyl-silyl (Me-TMSi) derivatives of the samples were

prepared as described previously (Barrow & Taylor 1987) and then reconstituted in 200 μl chloroform/hexane (4:1) before injection into a gas chromatograph-mass spectrometer (GC-MS). In order to determine the 3-hydroxyoxylipin yield when this yeast was grown on YM agar, many agar plates were cultivated until 1 Kg of wet biomass was recovered. These were subjected to oxylipin extraction as described before.

Electron impact (EI) mass spectra

EI mass spectra of the 3-hydroxy fatty acids were recorded on a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard 5972 MSD at 1447 EM V. A HP-5-60 m fused silica capillary column (0.25 mm i.d. and 0.25 μm coating thickness), with helium as carrier gas at 90 kPa head pressure, was used at an initial oven temperature of 110°C, the latter was increased by 6°C /min to a final temperature of 320°C.

Chemicals

All chemicals were obtained from reputable dealers.

3.1.4 Results and discussion

The results obtained in this study are depicted in Figures 1 – 3. When 1 Kg of wet biomass was subjected to hydroxyoxylipin extraction, as many as 5 g (SE < 5%) 3-hydroxyoxylipins could be extracted. Gas chromatography - mass spectrometry analysis showed that these oxylipins were 98% pure 3-hydroxy 16:0. Scanning electron micrograph studies showed that a web-like structure of threads are attached to the

vegetative cells (Fig. 1A,B). This structure turned out to mainly consist of 3-hydroxy palmitic acid (3-OH 16:0) as demonstrated by GC-MS analysis (Fig. 2A,B), since ethyl acetate extraction (see Materials and methods) abrogated the thread-like structure. The EI mass spectrum showed characteristic mass fragments *m/z* 175 [$CH_3O(CO).CH_2.CHO.TMSi$] indicative of 3-hydroxylation of the fatty acid and *m/z* 343 showing [M^+ - 15 (CH_3)] ion (Van Dyk et al. 1991).

After an extensive search, transmission electron microscopy showed that 3-OH 16:0 is attached to the cell wall surface of the vegetative cells (Fig. 3A-C). It is interesting to note the 3-hydroxyoxylipin-threads are present as micellar-like units characterized by an osmiophilic-hydrophilic outer layer and a more hydrophobic inner part.

It can be concluded from the results obtained in this study and from the previous literature that 3-OH oxylipins are apparently associated with structures that tend to aggregate. Consequently, a kind of adhesion property may be attributed to these compounds. Whether this occurs through entropic-based hydrophilic forces and/or hydrogen bonds is yet to be verified (Larsson 1994; Rudolph 1994). Moreover, the polar head of 3-hydroxy 16:0 is large in size compared with the hydrocarbon chain, hence the 3-hydroxyoxylipins are more likely to form thread-like micelles (Larsson 1994) which in turn may attach to the hydrophilic cell walls of the vegetative cells (Fig. 4).

It will be of interest to determine further 3-hydroxyoxylipin structures and their possible functional role in different fungi. Such structures may shed more light on the

evolutionary development of 3-hydroxyoxylipins and thereby assist in selecting fungi that can be used to produce novel oxylipins.

3.1.5 Acknowledgements

The authors wish to thank the Volkswagen Foundation, Germany (1/74643) and the National Research Foundation in South Africa for financial support.

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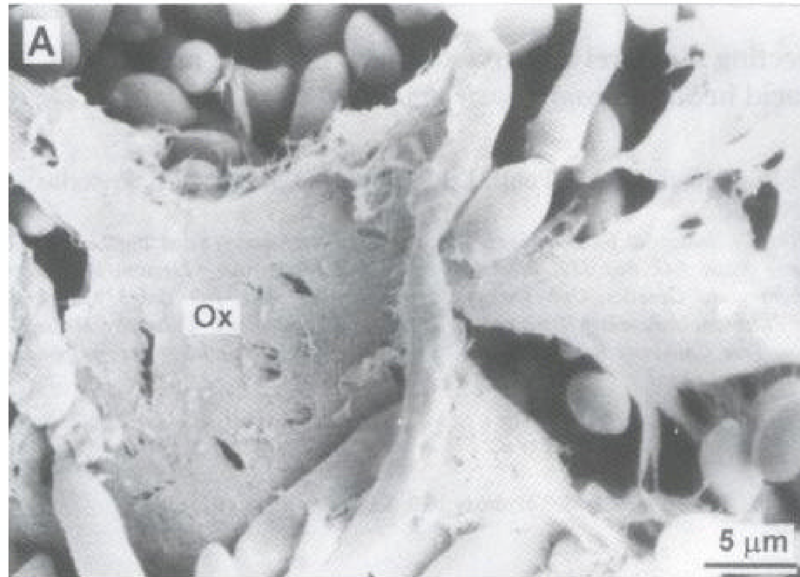

Figure 1A: Scanning electron micrograph showing thread-like oxylipins (Ox) attached to yeast cells in *S. malanga*.

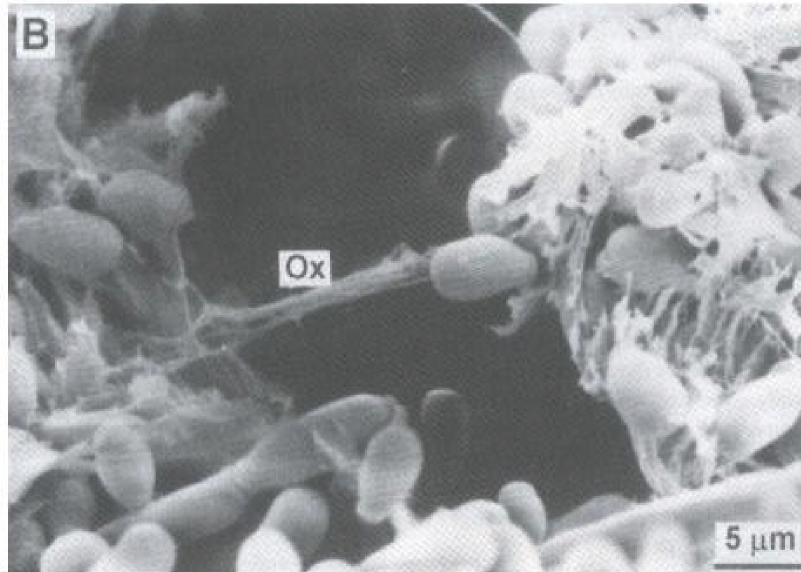

Figure 1B: Scanning electron micrograph showing thread-like oxylipins (Ox) attached to yeast cells in *S. malanga*.

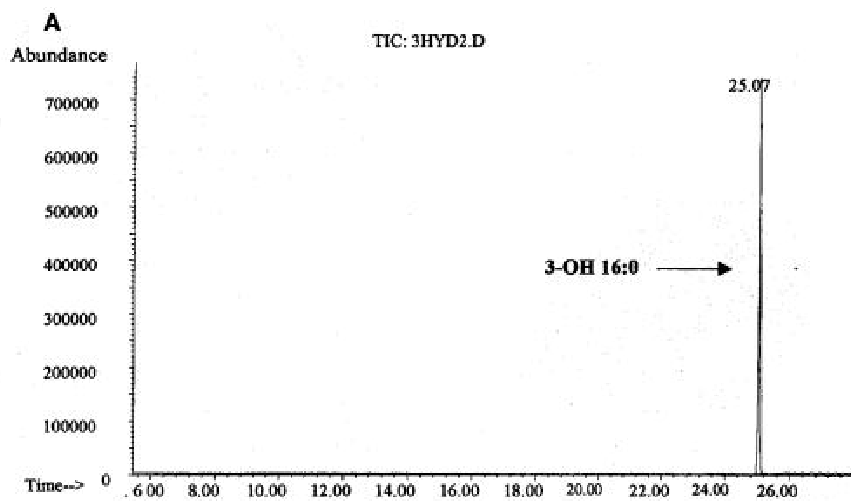

Figure 2A: Ion chromatogram of methyl-trimethylsilylated 3-OH 16:0 obtained from *S. malanga* when cultivated on solid medium.

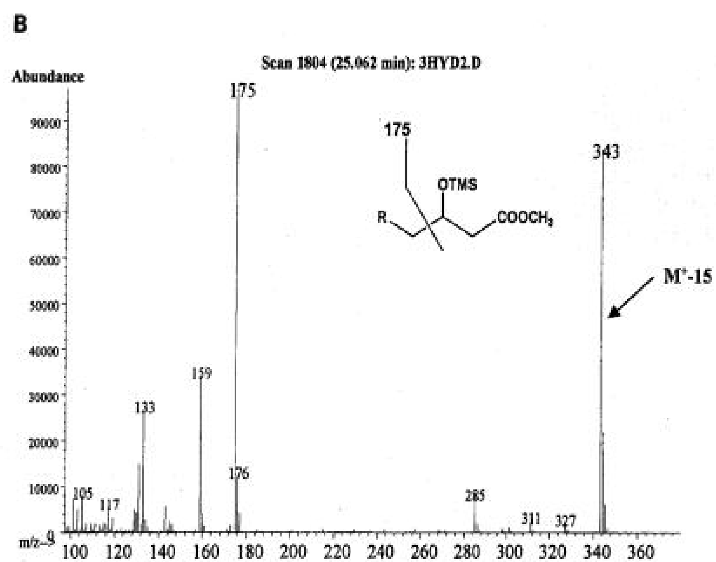

Figure 2B: Mass spectrum of methyl-trimethylsilylated 3-OH 16:0 obtained from *S. malanga* when cultivated on solid medium.

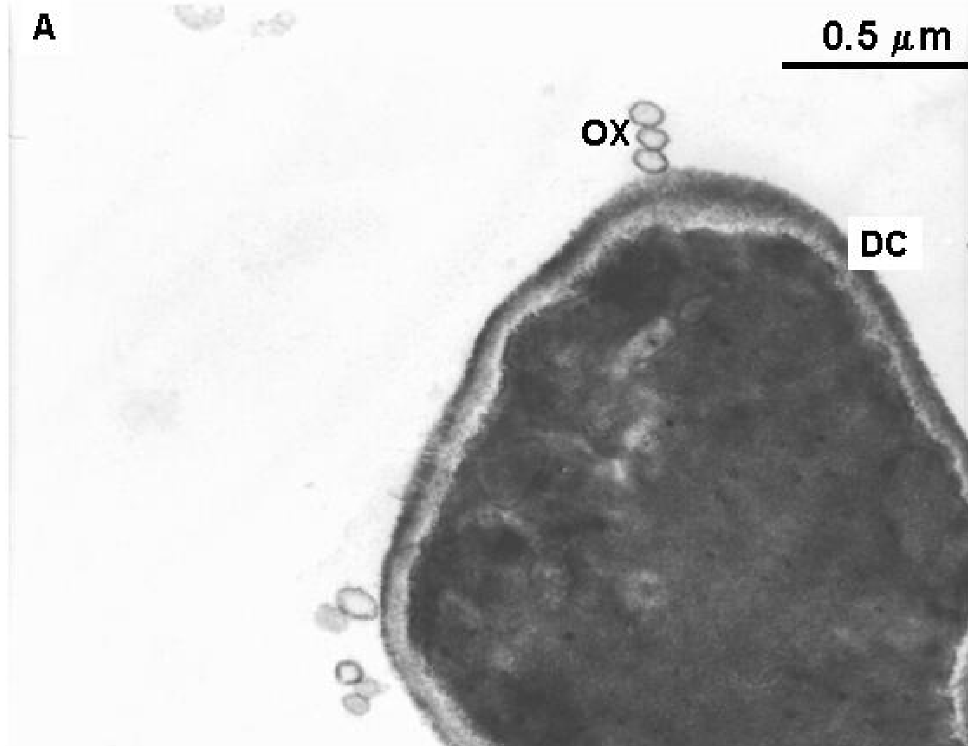

Figure 3A: Transmission electron micrograph showing oxylipins (Ox) attached to double layered cell walls of vegetative cells (DC) of *S. malanga*.

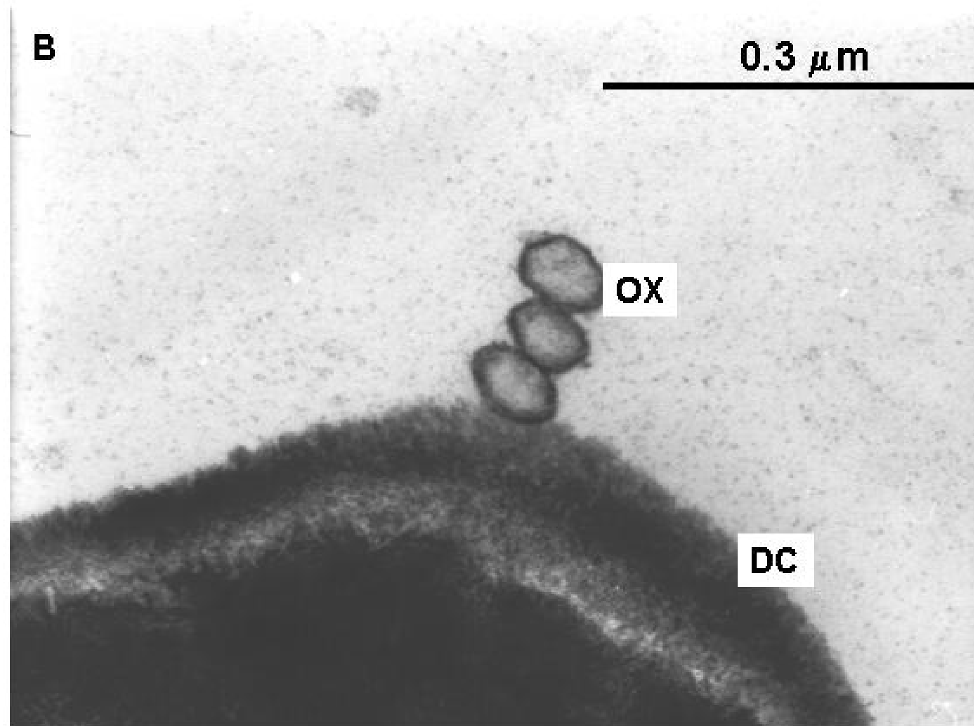

Figure 3B: Transmission electron micrograph showing oxylipins (Ox) attached to double layered cell walls of vegetative cells (DC) of *S. malanga*.

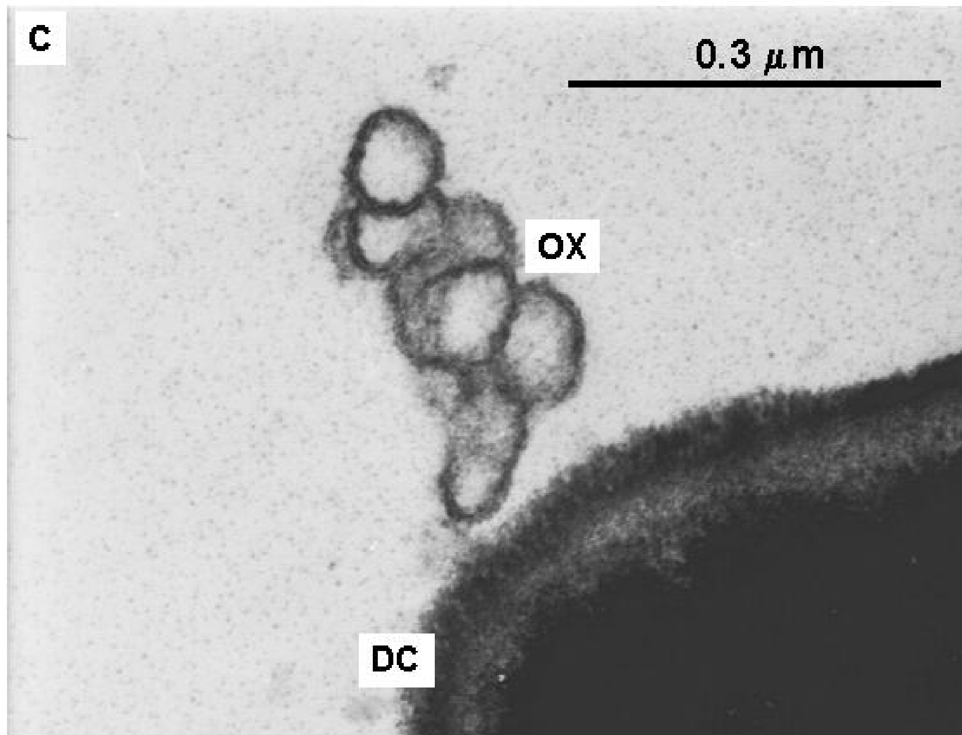

Figure 3C: Transmission electron micrograph showing oxylipins (Ox) attached to double layered cell walls of vegetative cells (DC) of *S. malanga*.

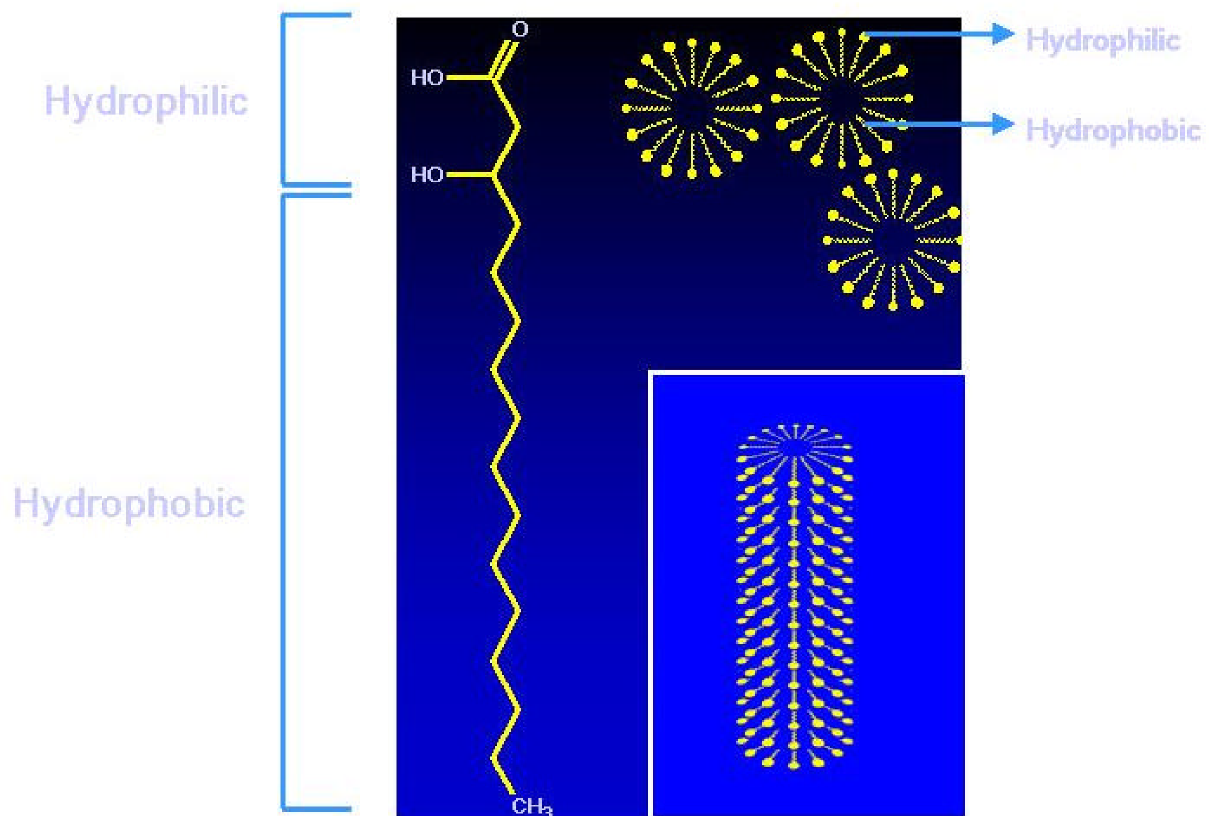

Figure 4: The schematic representation of a thread-like inverse micelle which may attach to the hydrophilic cell walls of the vegetative cells of *S. malanga*.

Section 3.2

Report on the discovery of a novel 3-hydroxy oxylipin cascade in the yeast *Saccharomycopsis synnaedendra*

This study has been published in Prostaglandins and Other Lipid Mediators **74:** 139-146 (2004).

Authors: Sebolai et al.

3.2.1 Abstract

A novel cascade of 3-hydroxy fatty acids was discovered in the yeast *Saccharomycopsis synnaedendra.* The cascade, probably derived from incomplete β-oxidation, comprises both even and uneven carbon numbered as well as saturated and unsaturated 3-hydroxy oxylipins. This yeast may now be used as model to further study the metabolism of these compounds as well as their biotechnological production.

Key words: Fungi; Gas chromatography - mass spectrometry; 3-Hydroxy oxylipin cascade; *Saccharomycopsis synnaedendra*; Yeasts

3.2.2 Introduction

3-Hydroxy oxylipins are oxygenated fatty acid derivatives that are produced probably via incomplete β-oxidation [1,2]. These oxylipins occur freely in nature, and their presence in fungi, particularly yeasts, has been established using immunofluorescence microscopy and gas chromatography - mass spectrometry (GC-MS) [3,4]. Over the years, literature has shown that 3-hydroxy oxylipins are mostly found in close association with surfaces of sexual spores and vegetative cells of fungi that tend to cluster or aggregate [5,6]. These compounds may play a role in affecting aggregation or adhesion of these structures through entropic-based hydrogen bonds and/or hydrophobic interactions [7]. In addition, these oxylipins showed potent biological activity in signal transduction processes in human neutrophils and tumour cells [8].

However, at present the availability and variety of these oxylipins are limited [4]. Consequently, bioprospecting studies are employed to select fungi capable of producing a wide variety of these compounds in significant amounts for use in biological research. The present study reports on the discovery of a novel cascade of 3-hydroxy oxylipins in the yeast *Saccharomycopsis synnaedendra* using gas chromatography-mass spectrometry (GC-MS).

3.2.3 Materials and methods

Strain used and cultivation

Saccharomycopsis synnaedendra CBS 6161 T, held at the University of the Free State was used in this study. This yeast was cultivated on yeast-malt (YM) agar [9] for seven days. Following this, the vegetative cells were scraped from YM plates and then subjected to oxylipin analysis.

Oxylipin extraction and analysis

This was carried-out as described [10]. Here, cells were suspended in 200 ml distilled water at pH 3. Oxylipins were extracted using 400 ml ethyl acetate with the eventual evaporation of the organic phase. Subsequently, the extracts were derivatized using diazomethane and *bis*- (trimethylsilyl) trifluoroacetamide (BSTFA) in the presence of pyridine and, reconstituted in chloroform:hexane (4:1, v/v) before injection into the GC-MS.

Gas chromatography - mass spectrometry

A Finnigan Trace Ultra gas chromatograph (San Jose, California), which is equipped with an HP-5-60 m capillary column (0.23 µm i.d. and 0.1 µm, coating thickness) coupled to a Finnigan Trace DSQ MSMS (San Jose, California) was used to record the electron impact mass spectra of 3-hydroxy oxylipins. A sample volume of 1 µl was injected at a split ratio of 1:50. Helium was used as a carrier gas at a constant flow of 1 ml.min^{-1}. The MS was auto-tuned to *m/z* 0-700. The temperature was programmed

to rise by 5 ^oC per min and to be held for 2 min from an initial temperature of 110 ^oC to a final temperature of 280 ^oC.

Chemicals used

All chemicals used were obtained from reputable dealers.

3.2.4 Results and discussion

A whole cascade of 3-OH oxylipins were uncovered in this yeast (335.7 mg/Kg wet cells, SE < 5%) and includes 3-OH 16:0, 3-OH 18:0, 3-OH 20:0 and novel 3-OH 17:0, 3-OH 18:1, 3-OH 19:0, 3-OH 19:1, and 3-OH 22:0 (Fig. 1) (Table 1). In all cases, their EI-MS spectra showed a base (major) peak at *m/z* 175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$] and other fragment ions denoting M^+ - CH_3 (Figs 2-9). This is in accordance with the structure elucidation of a typical 3-hydroxy oxylipin such as 3-hydroxy eicosatetraenoic acid (3-HETE) by Van Dyk et al. [11].

This is the first report on such an extensive cascade comprising of both saturated and unsaturated 3-hydroxy oxylipins. This now opens the way for further metabolic studies and research into the biological activities of this wide array of 3-hydroxy oxylipins.

3.2.5 Acknowledgements

The authors wish to thank National Research Foundation in South Africa and Volkswagen Foundation (1/74643) in Germany for financially supporting this study.

3.2.6 References

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Table 1: Oxylipins found in *Saccharomycopsis synnaedendra.*

3-Hydroxy metabolite	Pronounced peaks
3-OH 16:0	175; 343 [M^+-15 (CH_3)]
3-OH 17:0	175; 357 [M^+-15 (CH_3)]
3-OH 18:0	175; 371 [M^+-15 (CH_3)]
3-OH 18:1	175; 369 [M^+-15 (CH_3)]
3-OH 19:0	175; 385 [M^+-15 (CH_3)]
3-OH 19:1	175; 383 [M^+-15 (CH_3)]
3-OH 20:0	175; 399 [M^+-15 (CH_3)]
3-OH 22:0	175; 427 [M^+-15 (CH_3)]

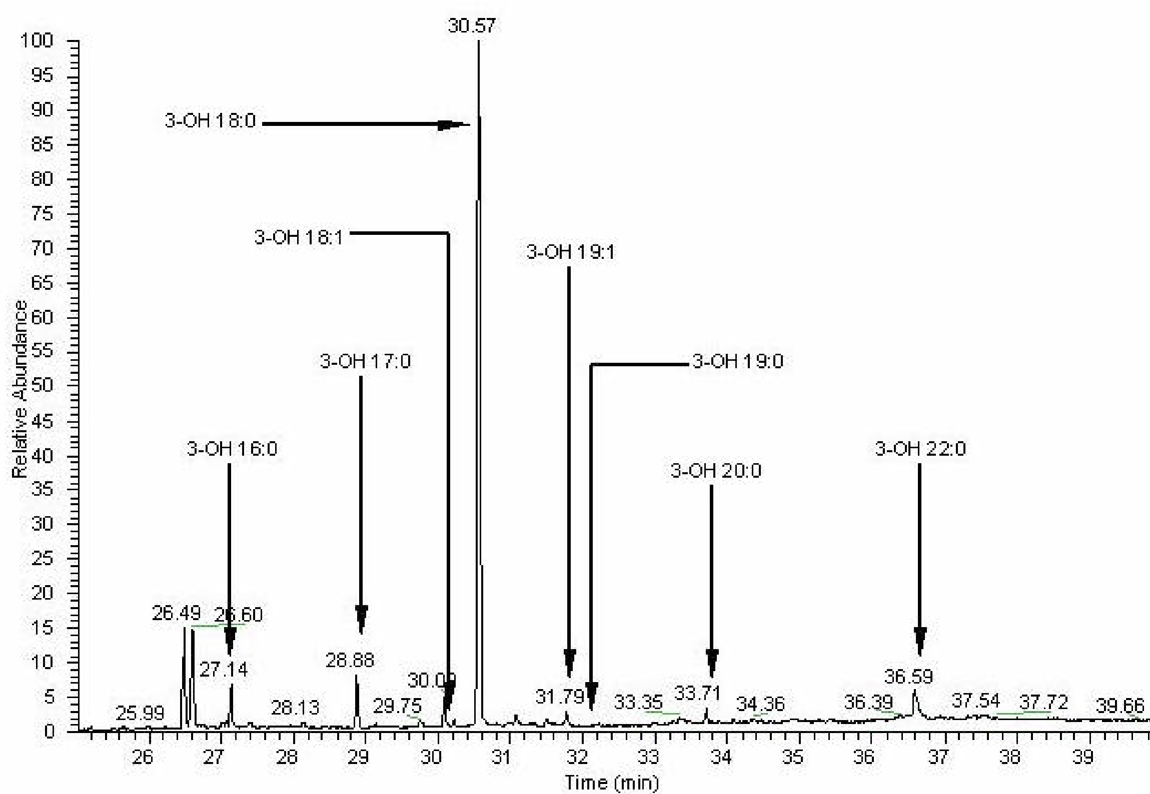

Figure 1: Total ion chromatogram of methyl-trimethylsilylated samples obtained from *Saccharomycopsis synnaedendra.*

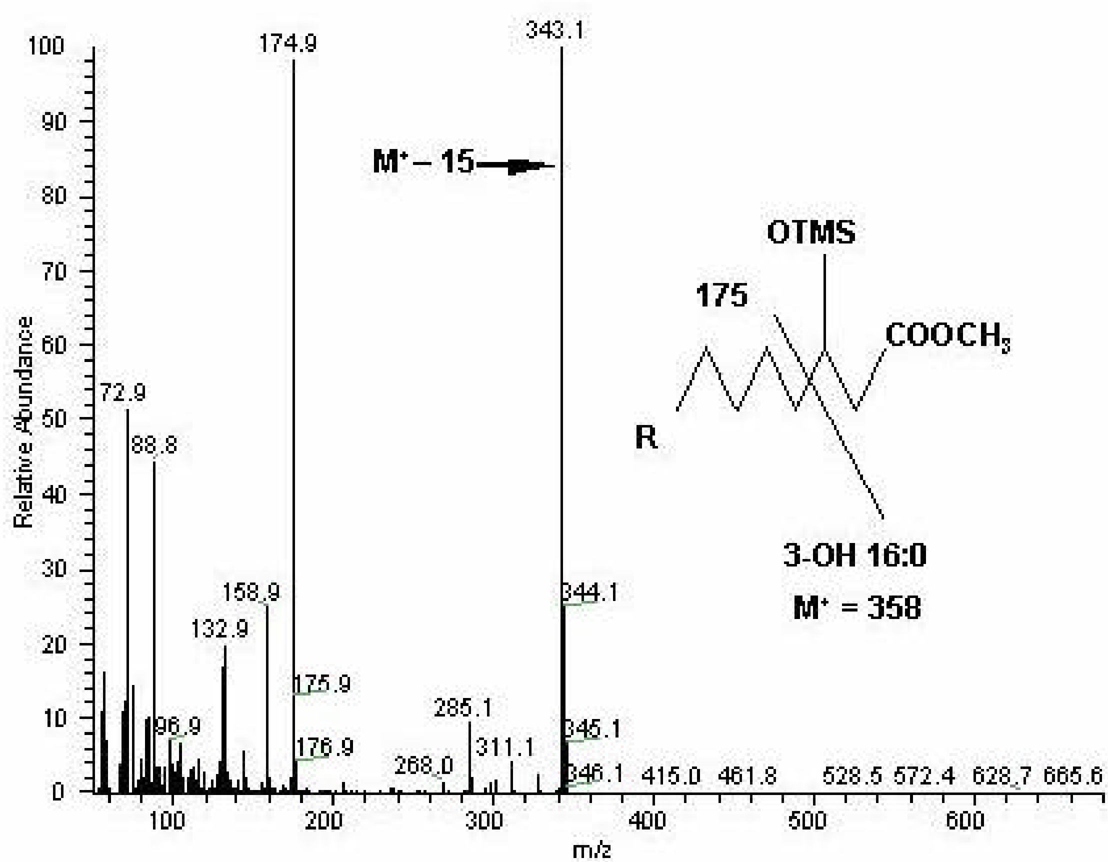

Figure 2: EI mass spectrum of methyl-trimethylsilylated 3-OH 16:0. The *m/z* 175 was derived from the esterified carboxyl end.

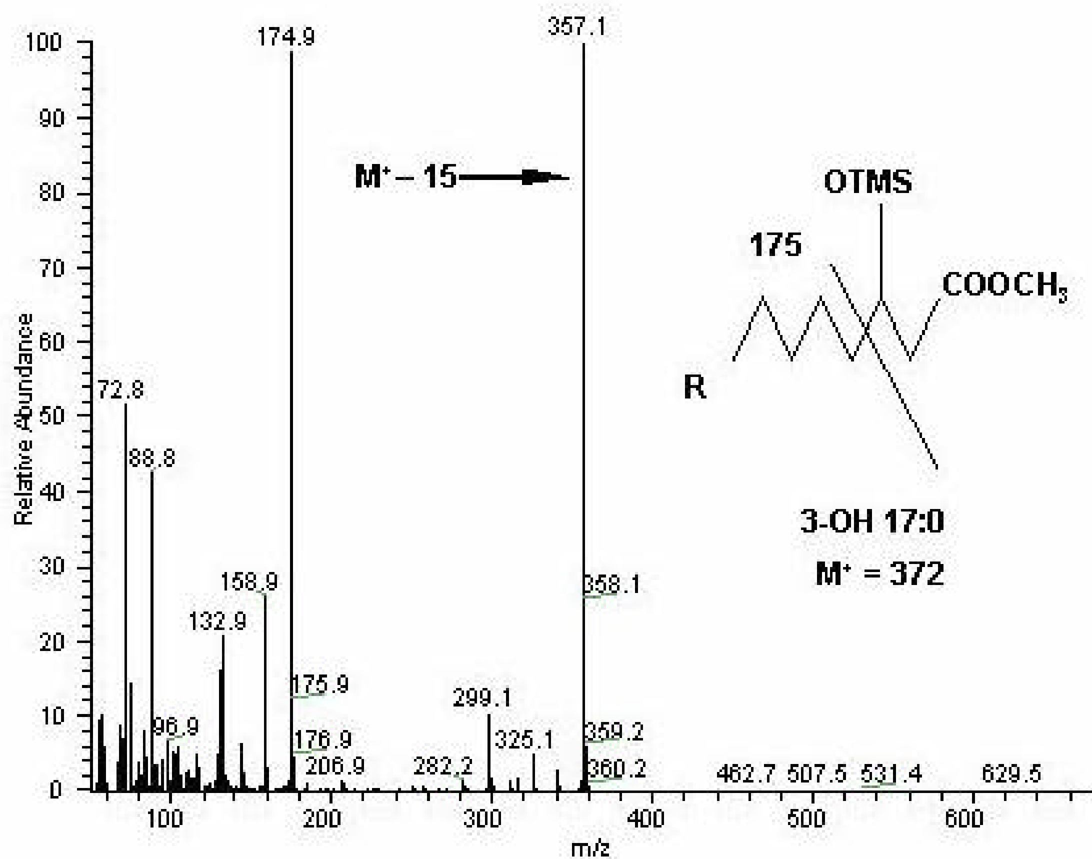

Figure 3: EI mass spectrum of methyl-trimethylsilylated 3-OH 17:0. The *m/z* 175 was derived from the esterified carboxyl end.

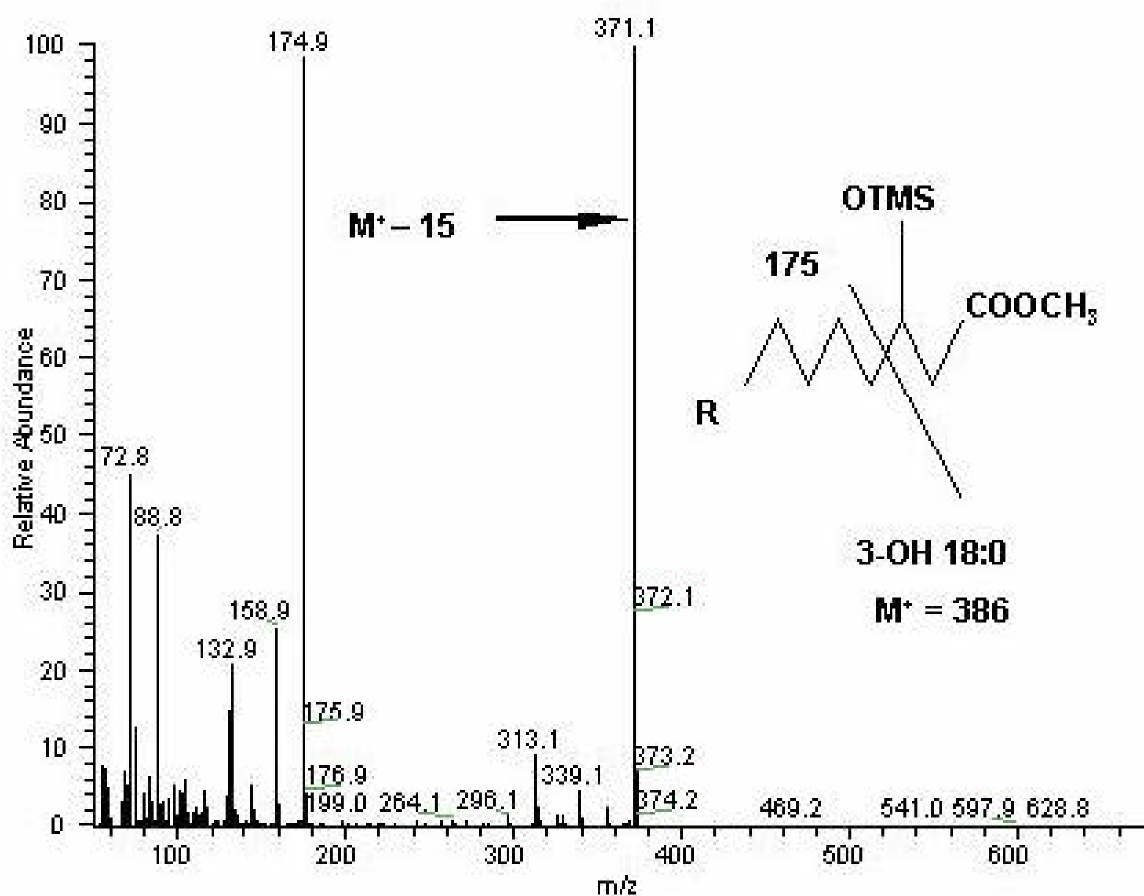

Figure 4: EI mass spectrum of methyl-trimethylsilylated 3-OH 18:0. The *m/z* 175 was derived from the esterified carboxyl end.

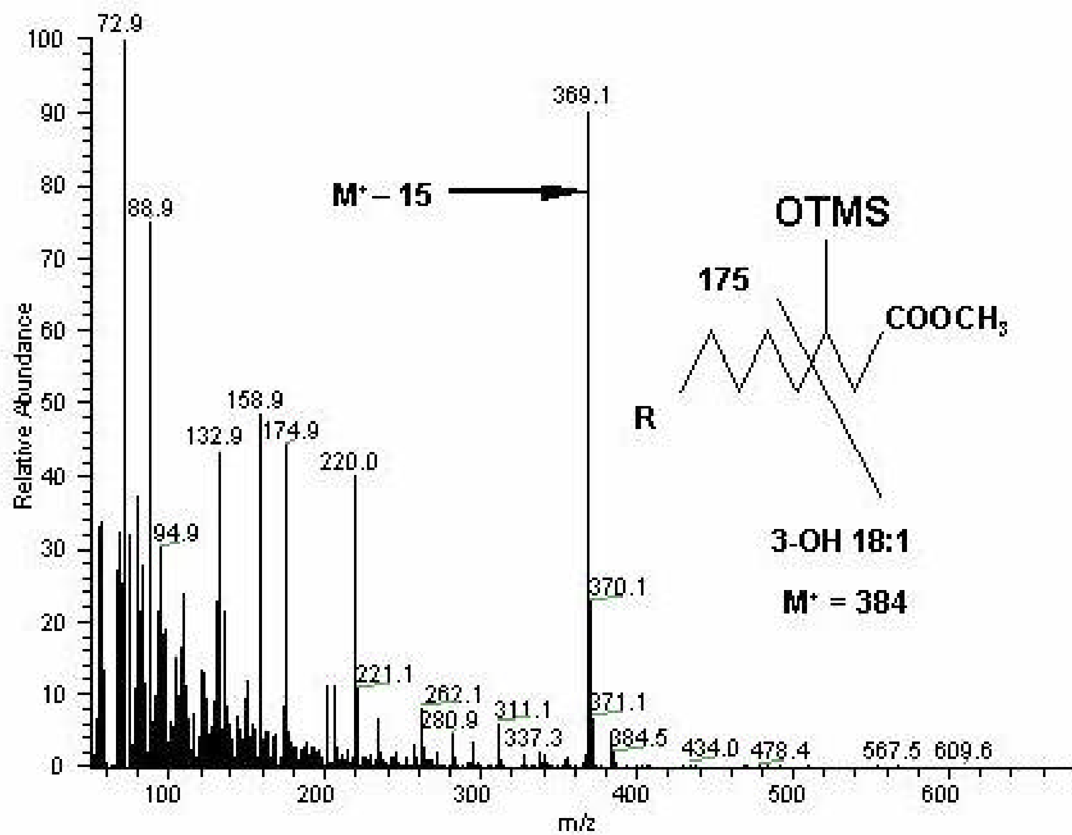

Figure 5: EI mass spectrum of methyl-trimethylsilylated 3-OH 18:1. The *m/z* 175 was derived from the esterified carboxyl end.

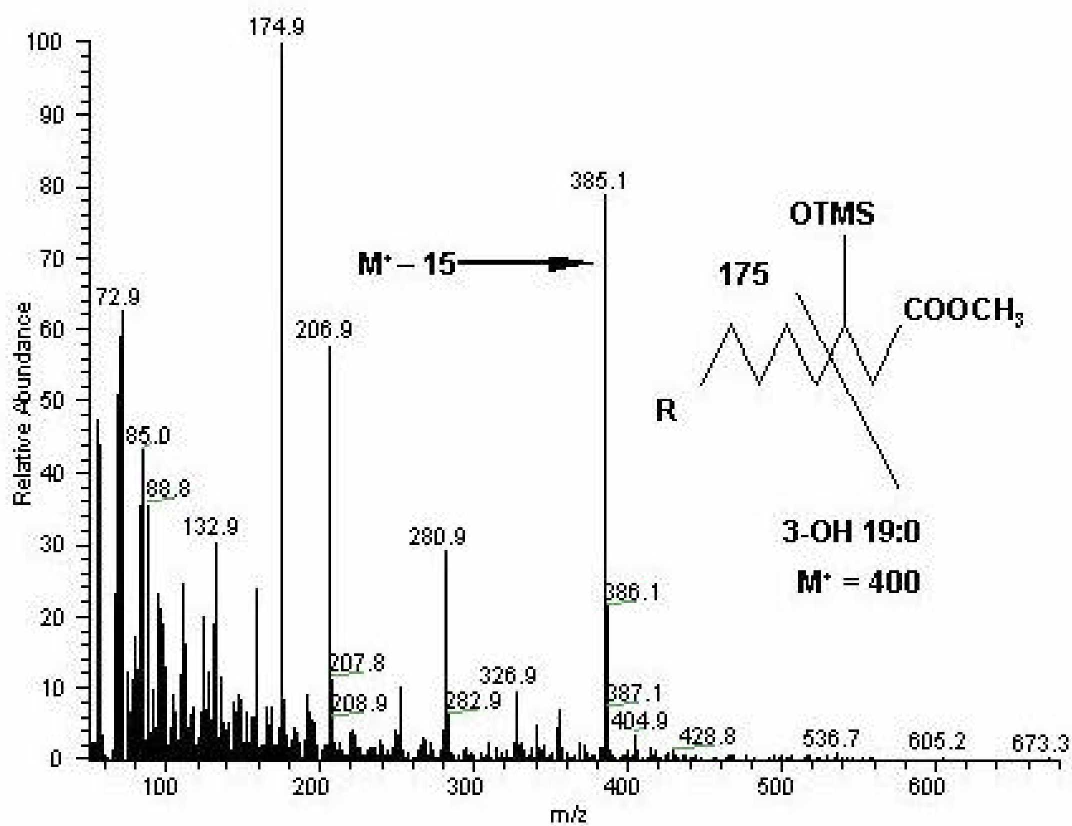

Figure 6: EI mass spectrum of methyl-trimethylsilylated 3-OH 19:0. The *m/z* 175 was derived from the esterified carboxyl end.

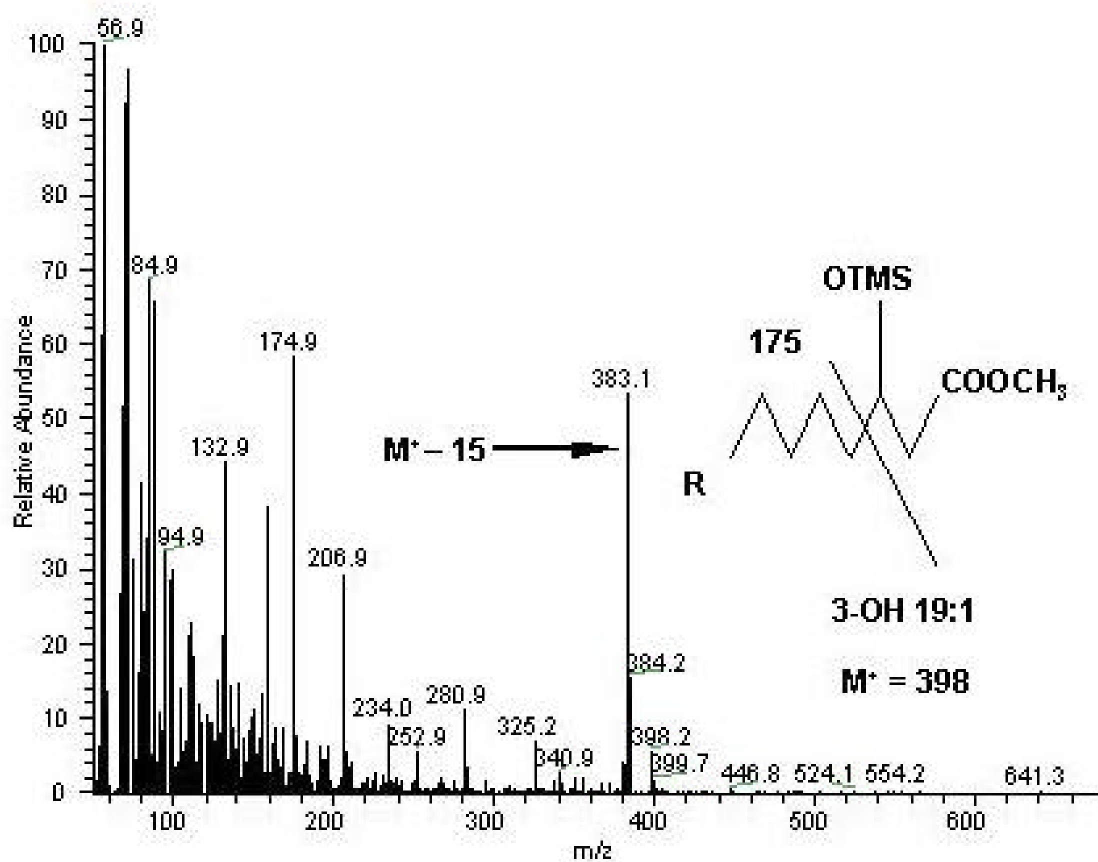

Figure 7: EI mass spectrum of methyl-trimethylsilylated 3-OH 19:1. The *m/z* 175 was derived from the esterified carboxyl end.

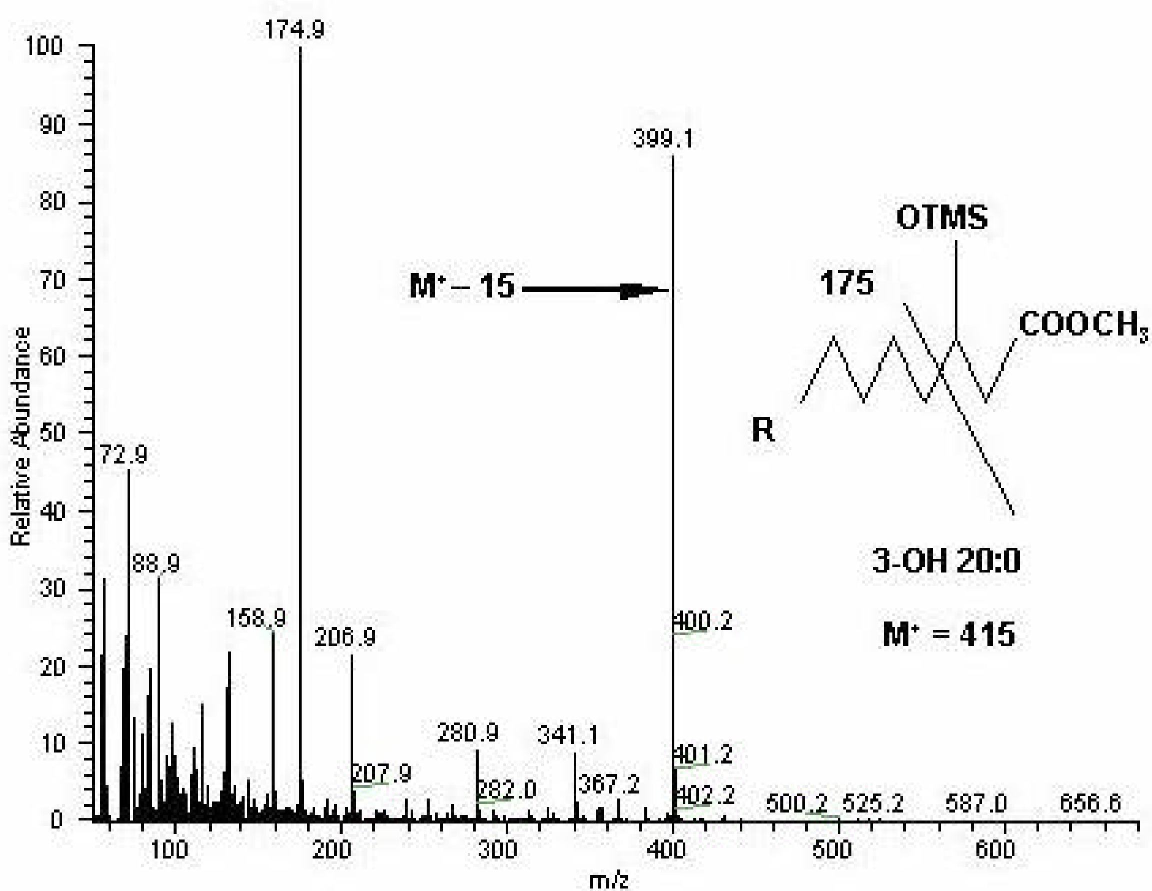

Figure 8: EI mass spectrum of methyl-trimethylsilylated 3-OH 20:0. The *m/z* 175 was derived from the esterified carboxyl end.

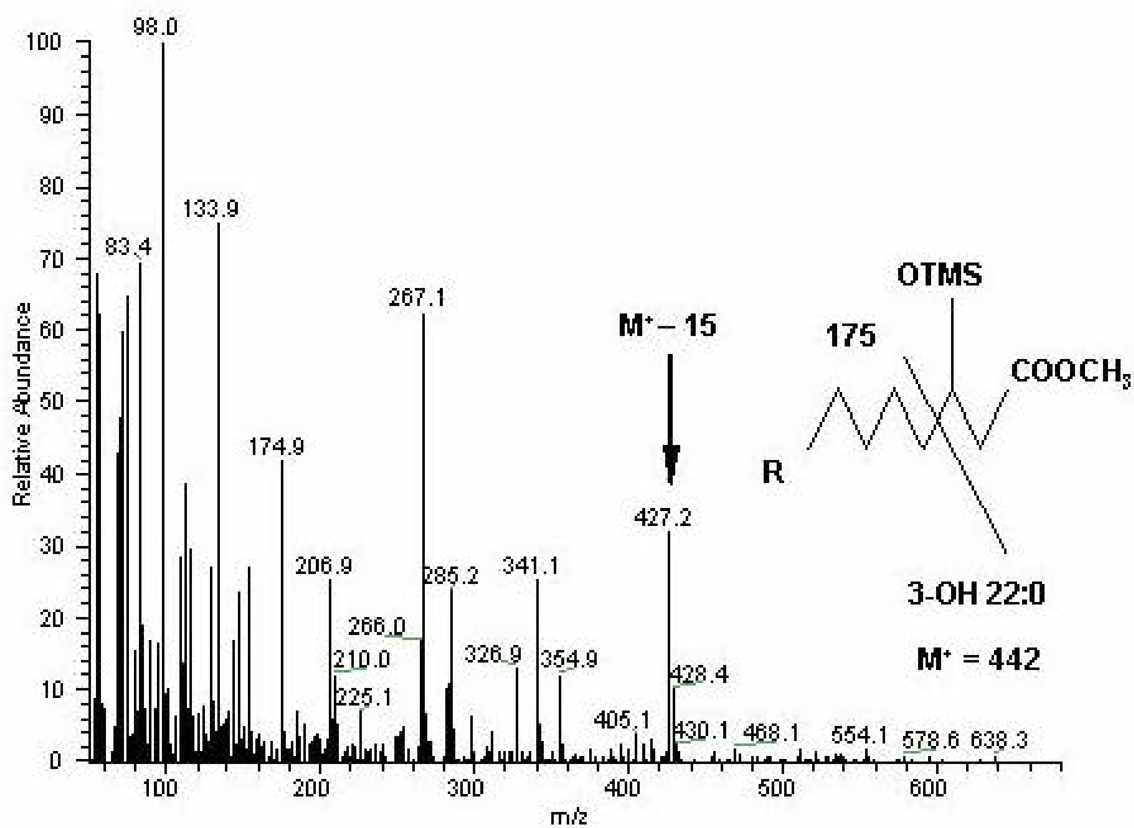

Figure 9: EI mass spectrum of methyl-trimethylsilylated 3-OH 22:0. The *m/z* 175 was derived from the esterified carboxyl end.

Section 3.3

The presence of an uneven carbon numbered 3-hydroxy oxylipin in the yeast *Saccharomycopsis capsularis*

This study has been submitted for publication to Systematic and Applied Microbiology (2004).

Authors: Sebolai et al.

3.3.1 Summary

The presence of a 3-hydroxy oxylipin was observed in close association with the aggregating ascospores of *Saccharomycopsis capsularis* through immunofluorescence microscopy. This was confirmed by gas chromatography - mass spectroscopy analysis where an uneven carbon numbered 3-OH 9:1 was detected.

Key words: Ascospores, 3-Hydroxy oxylipin, Immunofluorescence microscopy, *Saccharomycopsis capsularis,* Yeast

3.3.2 Introduction

Using immunofluorescence microscopy and gas chromatography - mass spectrometry, the presence of 3-hydroxy oxylipins in fungi, particularly in yeasts, has been established [5,8,9,12]. In most studies, these compounds have been reported to be associated with the aggregating sexual spores [10,11]. Interestingly, these compounds have also been found to be associated with the vegetative cells of *Saccharomyces cerevisiae* and *Saccharomycopsis malanga* [5,8]. In the latter yeast, these compounds formed web-like structures around the vegetative cells as observed by electron microscopy [8].

In this study we report on the presence of a 3-hydroxy oxylipin in *Saccharomycopsis capsularis* using immunofluorescence microscopy and gas chromatography - mass spectroscopy (GC-MS) analysis.

3.3.3 Materials and methods

Strain used and cultivation

S. capsularis UOFS Y-0447 T, held at the University of the Free State, was used in this study. This yeast was cultivated on YM (yeast-malt) agar [13] at 25 ^{o}C until sexual stage was reached. These cells were scraped from YM medium and treated for immunofluorescence microscopy and oxylipin analysis.

Immunofluorescence (IF) microscopy

Antibody preparation: antibodies against chemically synthesised 3-*R* HETE [1] were raised in a rabbit and were characterised by determining its titer, sensitivity and specificity [4].

Immunofluorescence protocol: This was performed as described [4]. In short, a loopful of yeast cells was suspended in 2 ml plastic tubes. This was followed by subsequent treatment with the primary antibody, and secondary antibody i.e. anti rabbit IgG coupled to FITC. After adequate washing, the cells were then placed on microscopic slides and photographed using a Nikon 2000 Confocal Laser Scanning Microscope (Japan). Appropriate controls were used for comparison. In addition, corresponding light micrographs were taken.

3-Hydroxy oxylipin extraction and analysis

This was performed as described [6]. In short, cells at sexual stage were suspended in 200 ml distilled water after which the pH was decreased to pH 4 by the addition of 3 % formic acid. Subsequently, oxylipins were extracted by using 2X volume (200 ml) of ethyl acetate followed by evaporation of the organic phase. Following this, samples were methylated and silylated (Me-TMSi) using diazomethane and *bis*-(trimethylsilyl) trifluoroacetamide (BSTFA) and then reconstituted in 200 µl chloroform:hexane (4:1, v/v) before injection into GC-MS for analysis.

Gas chromatography – mass spectrometry (GC-MS)

A Finnigan Trace Ultra gas chromatograph (San Jose, California) was used to record the EI mass spectrum of the 3-hydroxy oxylipin. This instrument is equipped with a Finnigan Trace DSQ MSMS (San Jose, California), with an HP-5-60 m fused silica capillary column (0.23 μm i.d. and 0.1 μm coating thickness). Helium was used as a carrier gas at a constant flow of 1 ml/min. The initial oven temperature of 110 ^{o}C was held for 2 min. before it was increased by 5 ^{o}C/min. to a final temperature of 280 ^{o}C. The MS was auto-tuned to *m/z* 50-500. A sample volume of 1 μl was injected at an inlet temperature of 230 ^{o}C at a split ratio of 1:50.

Chemicals used

Chemicals used in this study were of highest purity grade and were obtained from reputable dealers.

3.3.4 Results and discussion

Through immunofluorescence microscopy a 3-hydroxy oxylipin was found to be in close association with the surfaces of aggregating spheroidal ascospores of the yeast *S. capsularis* (Fig. 1). Similar results were reported in *Dipodascus* [10] and the Lipomycetaceae [11].

The presence of this oxylipin was confirmed using GC-MS analysis (Fig. 2A, B). Here, we found a mono-unsaturated 3-OH 9:1, comprising an uneven number of

carbons, to be produced by this yeast (Table 1). This oxylipin produced a pronounced peak of *m/z* 175 [$CH_3O(CO).CH_2.CHO.TMSi$] in its mass spectrum, which is characteristic of 3-OH oxylipins [12]. This oxylipin is probably involved in the aggregation of these sexual spores through hydrogen bonds/or entropic-based hydrophobic interactions [7].

To conclude, it is interesting to note that an oxylipin with an uneven number of carbons was detected in this yeast. This is contrary to previous results where only oxylipins with an even carbon number were reported [2,8]. Strikingly, 3-OH 9:1 was also found in the zygomycotan fungus, *Pilobolus* [3]. The metabolism of these oxylipins in this genus should now be investigated.

3.3.5 Acknowledgements

The authors wish to thank the National Research Foundation in South Africa and Volkswagen Foundation, Germany (1/74643) for financial support.

3.3.6 References

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Table 1: Oxylipin found in *Saccharomycopsis capsularis.*

Metabolite	Fragment
3-OH 9:1	175; 243 [M^+-15 (CH_3)]

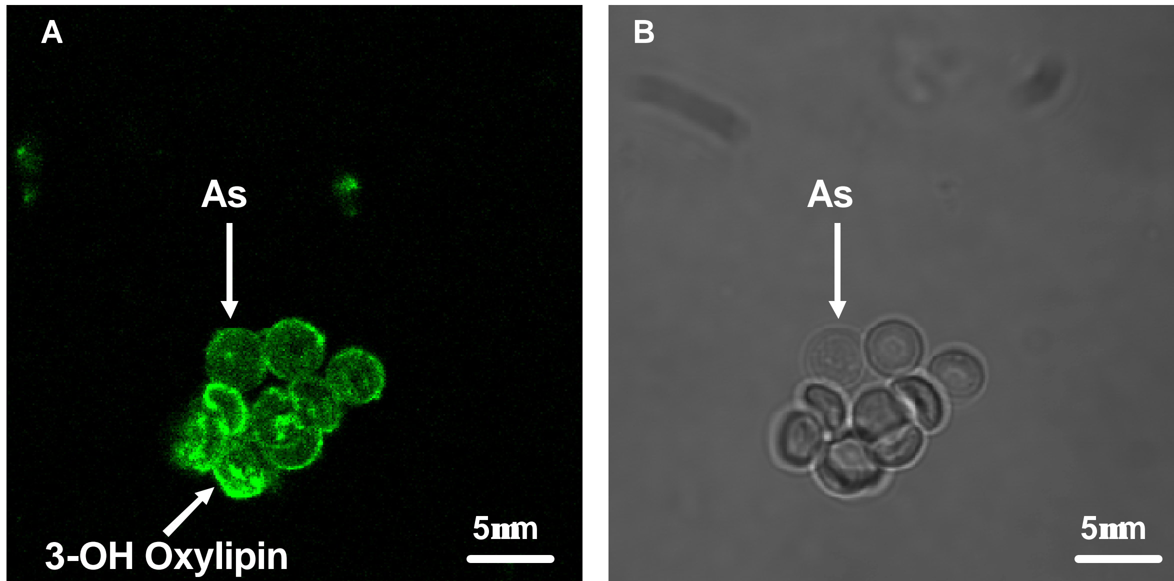

Figure 1: Immunofluorescence (A) and corresponding light micrograph (B) showing a 3-hydroxy oxylipin in close association with the surfaces of aggregated ascospores (As) of *S. capsularis*.

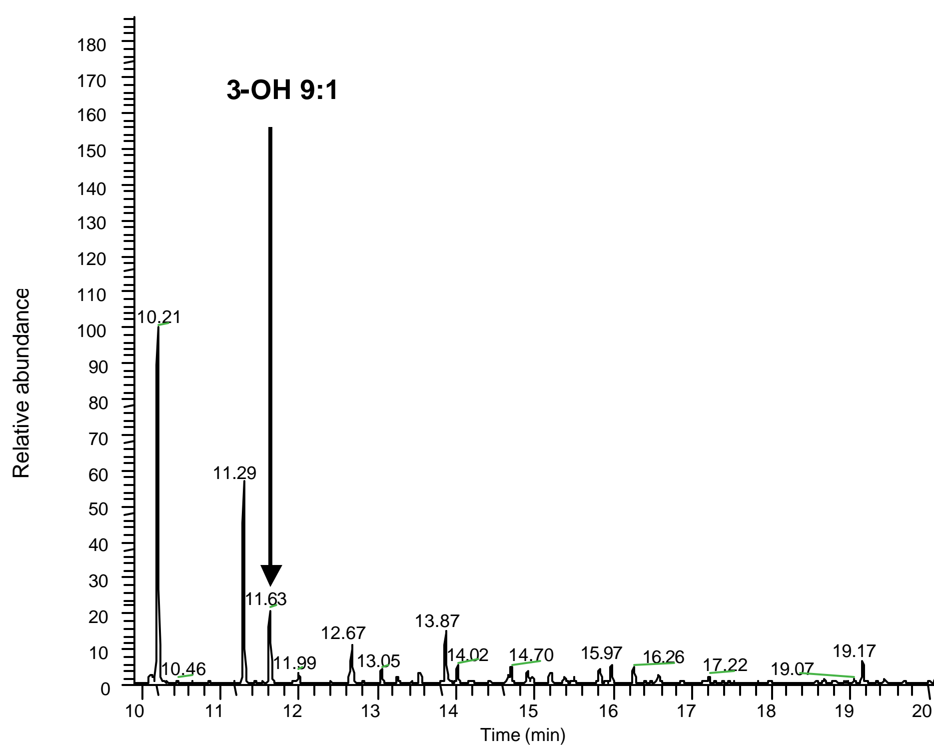

Figure 2A: Ion chromatograph of the methyl-trimethylsilylated sample of *S. capsularis.*

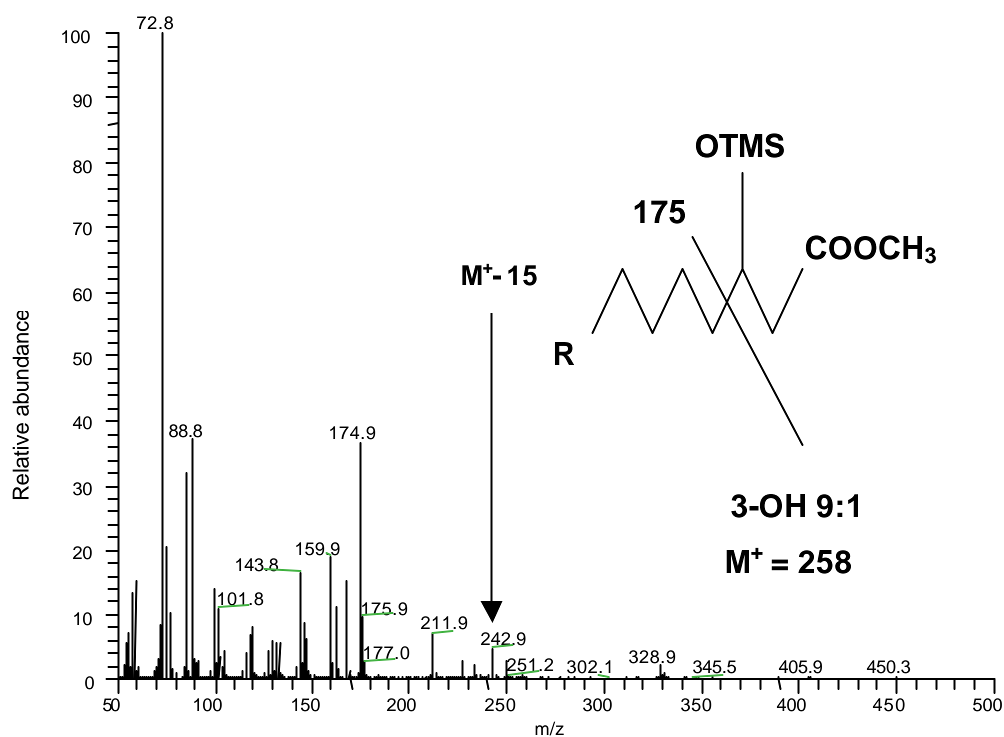

Figure 2B: Electron impact (EI) mass spectrum of methyl-trimethylsilylated 3-OH 9:1 obtained from *S. capsularis.*

Section 3.4

The presence of 3-hydroxy oxylipins on the ascospore surfaces of some species representing *Saccharomycopsis* Schiönning

This study has been submitted for publication to Canadian Journal of Microbiology (2004).

Author: Sebolai et al.

3.4.1 Abstract

Through gas chromatography – mass spectrometry, the presence of mainly 3-hydroxy 9:1 and 3-hydroxy 10:1 was detected in *Saccharomycopsis fermentans, S. javanensis* and *S. vini*. The distribution of these compounds was mapped using immunofluorescence microscopy and found to be closely associated with the surfaces of aggregating ascospores.

Key words: Ascospores, Gas chromatography–mass spectrometry, 3-Hydroxy oxylipins, Immunofluorescence microscopy, *Saccharomycopsis*, Yeast

3.4.2 Note

The presence of unsaturated and saturated 3-hydroxy fatty acids (oxylipins) in fungi, particularly yeasts, has been documented using immunofluorescence microscopy and gas chromatography - mass spectrometry (Van Dyk et al. 1991; Kock et al. 2000; Kock et al. 2003; Sebolai et al. 2001). These oxylipins are reported to be produced via incomplete β-oxidation (Van Dyk et al. 1994; Venter et al. 1997). Strikingly, these compounds have been found in close association with the aggregated sexual spores as well as aggregating vegetative cells of yeasts (Kock et al. 2000; Sebolai et al. 2001; Smith et al. 2003). Furthermore, 3-hydroxy oxylipins probably act as lubricant during ascospore release in some yeasts (Kock et al. 2004).

The present study reports on the presence of various 3-hydroxy oxylipins on the surfaces of ascospores of the yeast species *Saccharomycopsis fermentans, S. javanensis* and *S. vini*. This was detected using immunofluorescence microscopy and gas chromatography - mass spectrometry (GC-MS).

Saccharomycopsis fermentans CBS 7830, *S. javanensis* CBS 2555 and, *S. vini* CBS 4110, held at the University of the Free State were used in this study. These yeasts were cultivated on YM (yeast-malt) agar (Wickerham 1951) at 25 ^{o}C until sporulation was observed. Next, the sporulating cells with liberated spores were scraped from the YM plates and subjected to immunofluorescence microscopy and chemical oxylipin analysis.

Antibodies against chemically synthesised 3-*R* HETE (Bhatt et al. 1998) were raised in rabbits and then characterised by determining its titer, sensitivity and specificity (Kock et al. 1998).

Immunofluorescence protocol was carried out as described by Kock et al. (1998). In brief, a loopful of yeast cells was suspended in 2 ml plastic tubes for cell structure maintenance. Subsequently, the cells were treated with the primary antibody, and secondary antibody i.e. FITC anti-rabbit IgG. Following adequate washing, microscopic slides showing fluorescence were photographed using a Nikon TE 2000 Confocal Laser Scanning Microscope (Japan). Corresponding light micrographs were taken. In addition, appropriate controls were used for comparison.

Extraction of oxylipins was carried out as described (Nigam 1987). In brief, the cells were suspended in 200 ml distilled water at pH 3. The oxylipins were then extracted using 2X volume (400 ml) of ethyl acetate, which was finally evaporated under a stream of nitrogen. Subsequently, the extracts were methylated and silylated (Me-TMSi) using diazomethane and *bis*- (trimethylsilyl) trifluoroacetamide (BSTFA) respectively, - the latter in the presence of pyridine and, eventually reconstituted in chloroform:hexane (4:1, v/v) before injection into the GC-MS.

A Finnigan Trace Ultra gas chromatograph (San Jose, California), which is equipped with an HP-5-60 m capillary column (0.23 µm i.d. and 0.1 µm, coating thickness) coupled to a Finnigan Trace DSQ MS-MS (San Jose, California) was used to

record the electron impact (EI) mass spectra of 3-hydroxy oxylipins. A sample volume of 1 µl was injected at an inlet temperature of 230 ^{o}C at a split ratio of 1:50. Helium was used as a carrier gas at a constant flow of 1 ml.min^{-1}. The initial oven temperature of 110 ^{o}C was held for 2 min. before it was increased by 5 ^{o}C per min. to a final temperature of 280 ^{o}C. The MS was auto-tuned to *m/z* 0-400. All chemicals used in this study were obtained from major retailers.

The immunofluorescence microscopy results obtained in this study are shown in Figs 1-3. Here, we found fluorescence to be distributed on the surfaces of the aggregated ascospores of *S. fermentans*, *S. javanensis* and *S. vini*. In addition, in *S. vini* (Fig. 3) a fluorescing inter-spore matrix between the aggregating sexual spores as well as fluorescing protuberances were found in close association with the surfaces of aggregating spores (Fig. 3).

This fluorescing material was demonstrated to be 3-hydroxy oxylipins by GC-MS analysis. We detected a 3-hydroxy oxylipin in *S. fermentans* (structure not elucidated), 3-OH 9:1 in *S. javanensis* and 3-OH 9:1 as well as 3-OH 10:1 in *S. vini* (Figs 4-10).

The EI mass spectra of all these methylated and silylated 3-hydroxy oxylipins were characterized by a pronounced base peak of *m/z* 175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$], which is indicative of the hydroxylation of fatty acids on the β-carbon (Van Dyk et al. 1991). In *S. javanensis* and *S. vini*, the M^+ - 15 peak (characteristic of the loss of the methyl group usually attached to the carboxyl group)

was identified (Figs 5, 7, 9 and 11) (Table 1). It was however not possible to detect the mother ion in any of the yeasts studied which is in accordance with literature (Van Dyk et al. 1991).

To conclude, 3-hydroxy 9:1 was detected in *S. javanensis* and *S. vini* but not in *S. fermentans* which produces a novel, up till now, unidentified 3-hydroxy oxylipin. It is interesting to note that 3-hydroxy 9:1 was earlier identified in the mucoralean fungus, *Pilobolus* (Kock et al. 2001). The C9:1 fatty acid probably arises from the splitting of the C9-10 double bond of C18:3. This implies the presence of a monooxygenase or dioxygenase that hydroxylates the C9-10 double bond. It will be of interest, to map the variation of these compounds in this genus and to evaluate their taxonomic status.

3.4.3 Acknowledgements

The authors wish to thank the National Research Foundation in South Africa and the Volkswagen Foundation (1/74643) in Germany for financially supporting this study.

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Table 1: Oxylipins found in selected *Saccharomycopsis* species.

Species	Pronounced peak(s)	3-Hydroxy oxylipin
S. fermentans	175; ? [M^+-15 (CH_3)]	3-OH oxylipin
S. javanensis	175; 243 [M^+-15 (CH_3)]	3-OH 9:1
S. vini	175; 243 [M^+-15 (CH_3)]	3-OH 9:1
	175; 257 [M^+-15 (CH_3)]	3-OH 10:1

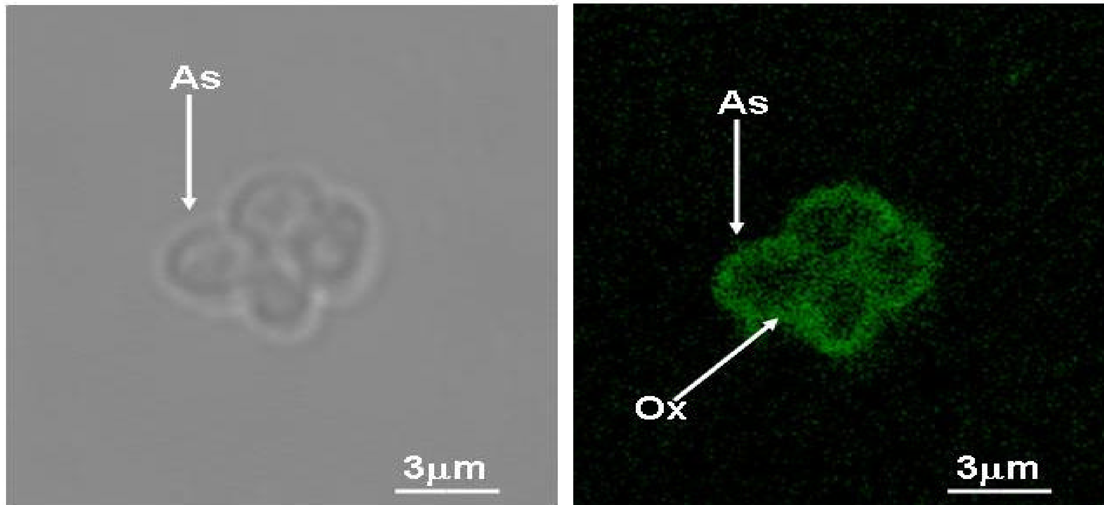

Figure 1: A light micrograph depicting the aggregated oblate-ovoidal ascospores (As) of *S. fermentans*, with the corresponding immunofluorescence micrograph showing the oxylipins (Ox) in close association with the surfaces of aggregated spores.

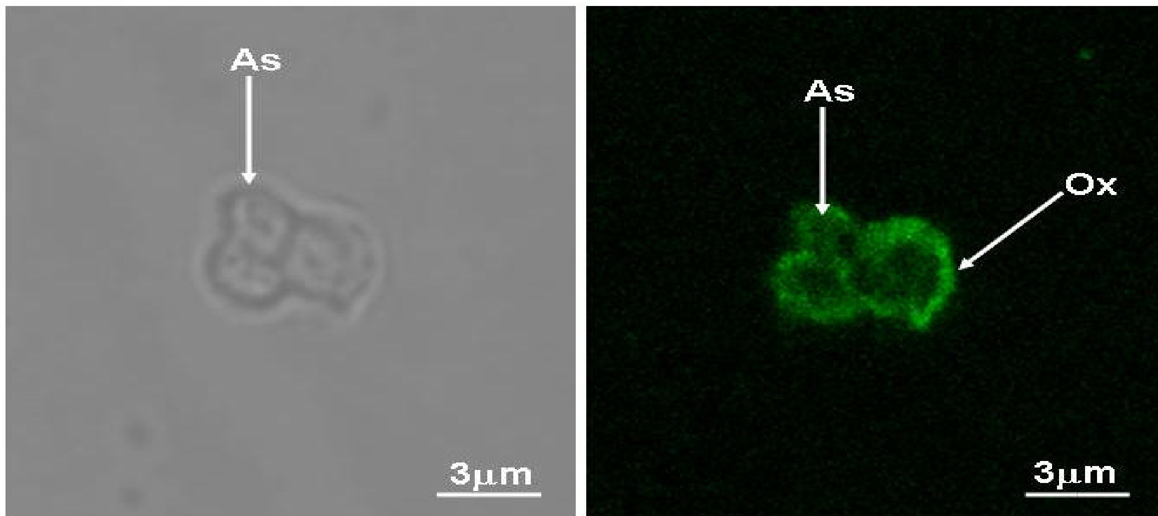

Figure 2: A light micrograph depicting the aggregated spheroidal ascospores (As) with faint equatorial ledges of *S. javanensis*, with corresponding immunofluorescence micrograph showing the oxylipins (Ox) in close association with the surfaces of aggregated spores.

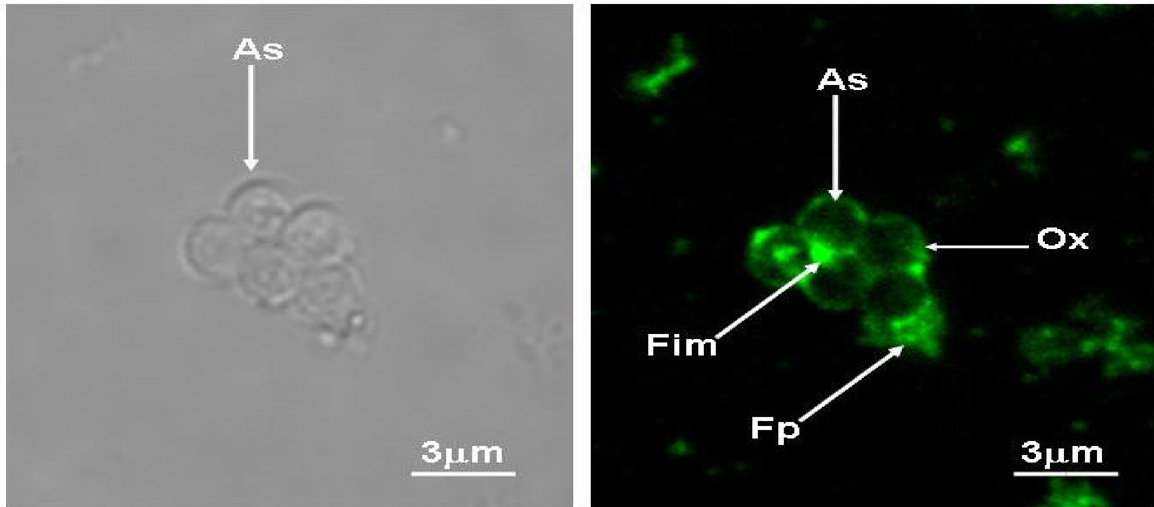

Figure 3: A light micrograph depicting the aggregated spheroidal ascospores (As) of *S. vini*, with corresponding immunofluorescence micrograph showing the oxylipins (Ox) in close association with the surfaces of aggregated spores. Fim = fluorescing inter-spore matrix, Fp = fluorescing protuberance.

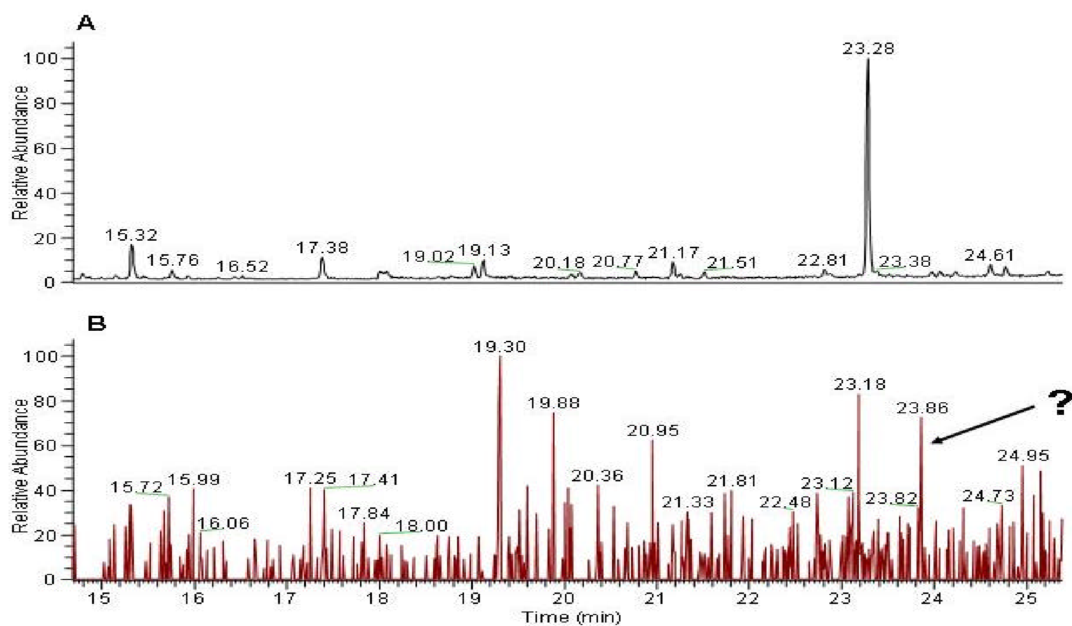

Figure 4: Ion chromatogram (A) and the specific ion extraction at *m/z* 175 (B) of *S. fermentans.*

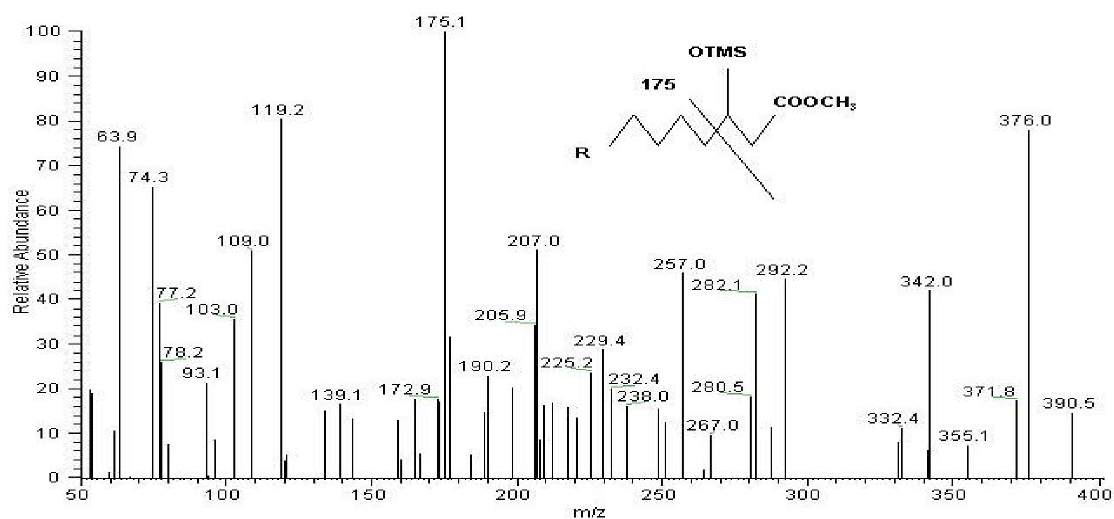

Figure 5: Electron impact mass spectrum of methyl-trimethylsilylated metabolite of *S. fermentans*. The *m/z* 175 was derived from the esterified carboxyl group.

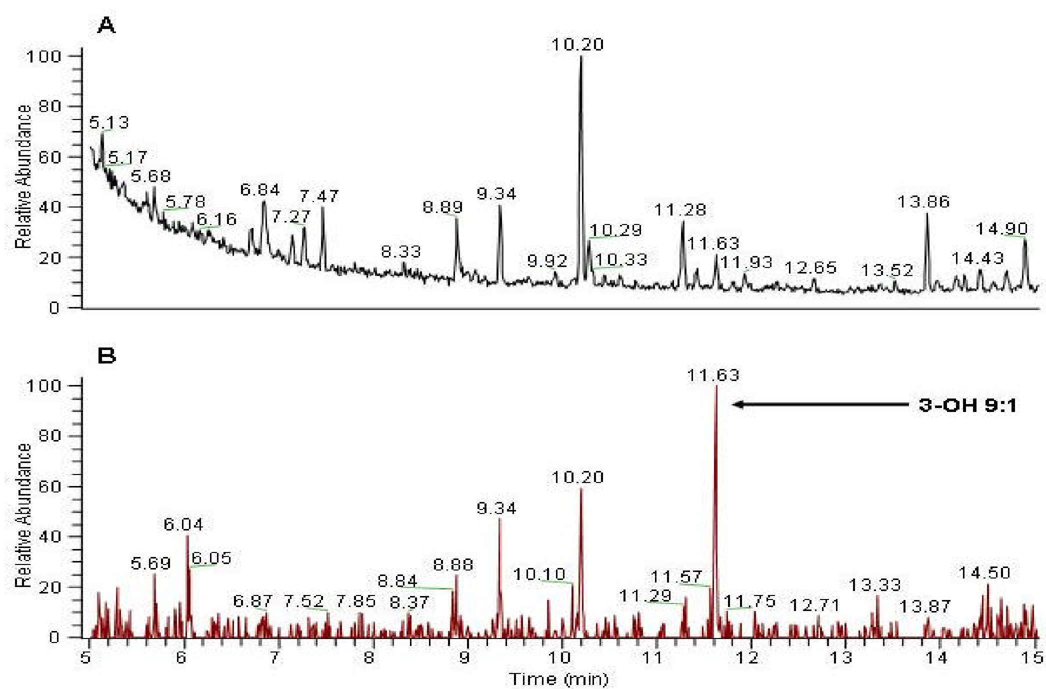

Figure 6: Ion chromatogram (A) of 3-OH 9:1 in *S. javanensis*, and the specific ion extraction at *m/z* 175 (B).

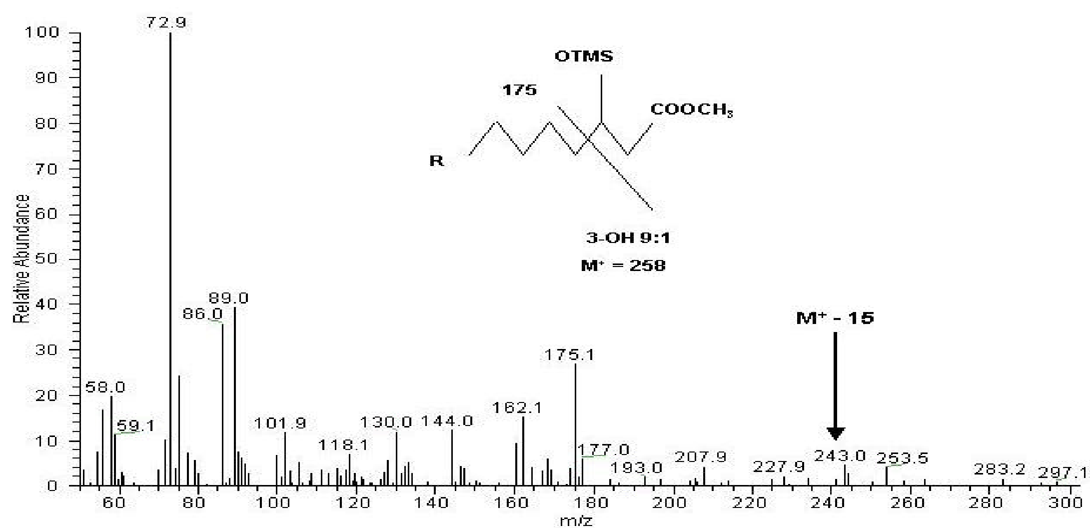

Figure 7: Electron impact mass spectrum of methyl-trimethylsilylated 3-OH 9:1 in *S. javanensis*. The *m/z* 175 was derived from the esterified carboxyl group.

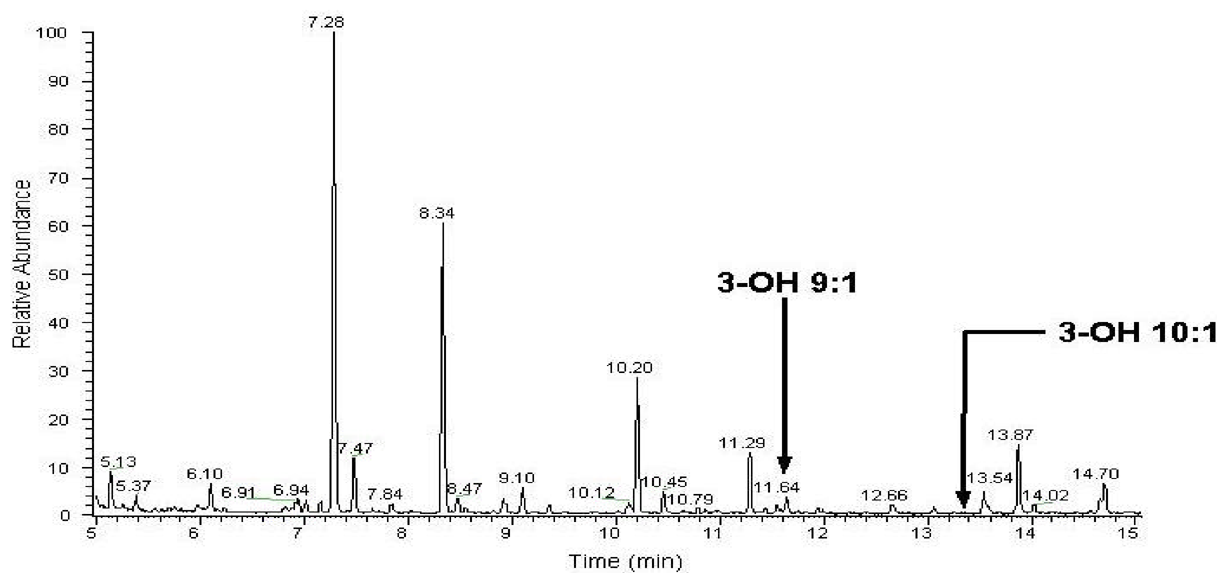

Figure 8: Ion chromatogram showing 3-OH 9:1 and 3-OH 10:1 obtained from *S. vini.*

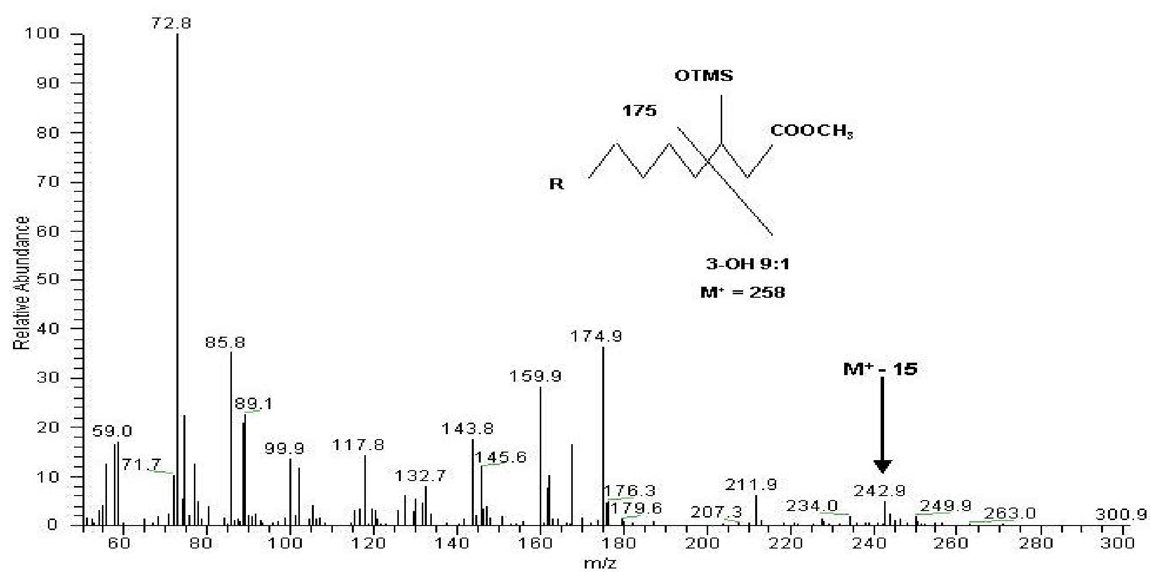

Figure 9: Electron impact mass spectrum of methyl-trimethylsilylated 3-OH 9:1 in *S. vini.* The *m/z* 175 was derived from the esterified carboxyl group.

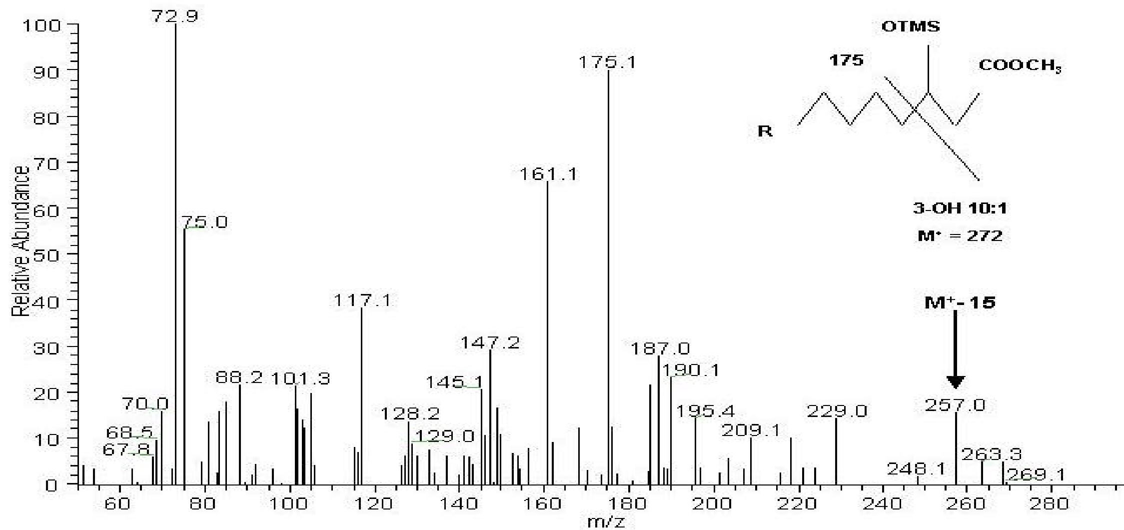

Figure 10: Electron impact mass spectrum of methyl-trimethylsilylated 3-OH 10:1 in *S. vini*. The *m/z* 175 was derived from the esterified carboxyl group.

Section 3.5

Conclusions

The current study demonstrates the presence of 3-hydroxy oxylipins within this genus. These compounds are probably produced via incomplete β-oxidation where 3-D hydroxyacyl-CoA is produced which cannot be or is poorly metabolised further by 3-hydroxyacyl-CoA dehydrogenase, and consequently accumulates inside the mitochondria (Finnerty 1989). This compound is then excreted as a 3-D hydroxy fatty acid (oxylipin). Interestingly, after conducting immunofluorescence microscopy studies, we found these compounds to be produced during the sexual stage and to be distributed on the surfaces of the aggregated sexual spores of *S. capsularis, S. fermentans, S. javanensis* and *S. vini*. These compounds were also found to be associated with the vegetative phases of *S. malanga* and *S. synnaedendra.*

The different 3-OH oxylipins that were uncovered in some species of this genus are summarized in Table 1. Following gas chromatography - mass spectrometry analysis, the oxylipin structures were found to be either saturated or unsaturated and comprise of even or uneven numbers of carbon atoms. These structures were characterised using mainly the 175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$] ion (hydroxylation of fatty acids on the β-carbon) and the M^+ - 15 ion (indicative of the loss of the methyl group). Importantly, a novel cascade of 3-hydroxy oxylipins in *S. synnaedendra* was uncovered. These findings suggest further investigation into the lipid metabolism of these medically important compounds as this may shed more light on their functional role in fungi and their possible biotechnological applications.

An interesting pattern is observed in this study regarding the type of oxylipin that is produced and the associated structures. Here, it was observed that short-chain 3-hydroxy oxylipins, in particular 3-OH 9:1, is mainly associated with the surfaces of aggregating ascospores, whereas, long chain 3-OH oxylipins (i.e. C_{16} - C_{22}) are mainly associated with the vegetative cells. It will be of interest to elucidate how the distribution pattern of these structurally diverse compounds within this genus plays a role in effecting aggregation or adhesion.

Based on the type of 3-hydroxy oxylipin produced it was possible to identify *S. fermentans* (novel unidentified 3-OH oxylipin), *S. malanga* (3-OH 16:0), *S. synnaedendra* (3-OH 16:0, 3-OH 17:0, 3-OH 18:0, 3-OH 18:1, 3-OH 19:0, 3-OH 19:1, 3-OH 20:0, 3-OH 22:0) and *S. vini* (3-OH 9:1, 3-OH 10:1). On the basis of this phenotypic characteristic it was not possible to distinguish between *S. capsularis* (3-OH 9:1) and *S. javanensis* (3-OH 9:1). The rest of the species of *Saccharomycopsis* should now be studied in this respect.

It is, however interesting to note that intracellular fatty acid composition could not be used as indicator to forecast the type of 3-OH oxylipins produced by these species. In fact, the fatty acids with uneven carbon atoms could not be detected intracellularly. It is concluded that the formation of 3-OH oxylipins with an uneven carbon atom number is probably not dependant on the fatty acids with even number of carbons esterified to the neutral-, glyco- and phospho-lipid fractions of these yeasts.

3.5.1 Reference

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Table 1: Summary of 3-OH oxylipins found in the genus *Saccharomycopsis* Schiönning.

Yeast	Peaks	3-Hydroxy oxylipin
S. capsularis	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	243 [M^+ - 15]	3-OH 9:1
S. fermentans	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	? [M^+ - 15]	3-OH metabolite
S. javanensis	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	243 [M^+ - 15]	3-OH 9:1
S. malanga	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	343 [M^+ - 15]	3-OH 16:0
S. synnaedendra	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	343 [M^+ - 15]	3-OH 16:0
	357 [M^+ - 15]	3-OH 17:0
	371 [M^+ - 15]	3-OH 18:0
	369 [M^+ - 15]	3-OH 18:1
	385 [M^+ - 15]	3-OH 19:0
	383 [M^+ - 15]	3-OH 19:1
	399 [M^+ - 15]	3-OH 20:0
	427 [M^+ - 15]	3-OH 22:0
S. vini	175 [$CH_3O(CO).CH_2.CHOSi(CH_3)_3$]	C-3 hydroxylation
	243 [M^+ - 15]	3-OH 9:1
	257 [M^+ - 15]	3-OH 10:1

SUMMARY

In this study, the construction of a forecasting model, using intracellular fatty acid composition as indicator, was attempted to assist in the search for yeasts capable of producing 3-hydroxy oxylipins. In order to achieve this, it was first attempted to establish a database mapping the distribution of fatty acids (FAs) associated with the neutral-, glyco- and phospholipid fractions of the 10 species representing the genus *Saccharomycopsis*. It was possible to identify nine of the 10 species i.e. *Saccharomycopsis capsularis, S. crataegensis, S. fibuligera, S. javanensis, S. malanga, S. schoenii, S. selenospora, S. synnaedendra* and *S vini* with the exception of *S. fermentans. Saccharomycopsis crataegensis* was unique since it produced by far the highest percentage neutral lipids (52.4% w/w) while *S. schoenii* produced the highest percentage phospholipids (35.9% w/w). All strains produced palmitic- (16:0), stearic- (18:0), oleic- (18:1) and linoleic acid (18:2) in all lipid fractions analysed. The major FAs produced were 18:1 and 18:2, while palmitoleic- (16:1) and α-linolenic acid [18:3 (ω-3)] varied between species. *Saccharomycopsis capsularis* produced the highest percentage 18:2 in the neutral lipid fraction while *S. crataegensis, S. malanga* and *S. selenospora* produced the highest percentages of 18:1, 18:0 and, 18:3 (ω-3) respectively, in the neutral lipids. *Saccharomycopsis vini* produced the lowest percentage 16:0 in this fraction. *Saccharomycopsis fibuligera* and *S. schoenii* produced the highest percentages of 16:0 and 18:2 respectively in the glycolipid fraction. *Saccharomycopsis javanensis* and *S. synnaedendra* produced the highest percentages of 18:1 and 16:1 respectively in the phospholipid fraction. Although it was possible to differentiate between most species

using this phenotypic character, these FAs could not be used to predict what kind of 3-OH oxylipins these species are capable of producing.

Saccharomycopsis fermentans (novel unidentified 3-OH oxylipin), *S. malanga* (3-OH 16:0), *S. synnaedendra* (3-OH 16:0, 3-OH 17:0, 3-OH 18:0, 3-OH 18:1, 3-OH 19:0, 3-OH 19:1, 3-OH 20:0, 3-OH 22:0) and *S. vini* (3-OH 9:1, 3-OH 10:1) could be separated using this character. Although, *S. capsularis* and *S. javanensis* both produced 3-OH 9:1, fatty acids with uneven carbon atoms which may serve as precursors could not be detected in the neutral-, glyco- or phospho-lipid fractions.

OPSOMMING

Hierdie studie het gepoog om ‘n voorspellingsmodel met intrasellulêre vetsuursamestelling as indikator, op te stel. Die doel hiervan was om behulpsaam te wees in die soeke na giste wat 3-hidroksievetsure kan produseer. Ten einde in die doel te slaag is eerstens gepoog om ‘n databasis van die verspreiding van vetsure geassosieer met die neutrale, gliko- en fosfolipiede van 10 spesies van die genus *Saccharomycopsis* op te stel. Dit was moontlik om nege van die 10 spesies te onderskei, nl. *Saccharomycopsis capsularis, S. crataegensis, S. fibuligera, S. javanensis, S. malanga, S. schoenii, S. selenospora, S. synnaedendra* en *S vini,* met die uitsondering van *S. fermentans. Saccharomycopsis crataegensis* was uniek aangesien dit verreweg die hoogste persentasie neutrale lipiede geproduseer het (52.4% w/w), terwyl *S. schoenii* die hoogste persentasie fosfolipiede geproduseer het (35.9% w/w). Alle stamme het palmitien- (16:0), stearien- (18:0), oleïn- (18:1) en linolieensuur (18:2) in alle geanaliseerde lipiedfraksies geproduseer. Die hoof vetsure was 18:1 en 18:2, terwyl palmitoleïn- (16:1) en α-linoleen [18:3 (ω-3)] tussen spesies gevarieer het. *Saccharomycopsis capsularis* het die hoogste persentasie 18:2 in die neutrale lipiedfraksie geproduseer, terwyl *S. crataegensis, S. malanga* en *S. selenospora* onderskeidelik die hoogste persentasies 18:1, 18:0 en 18:3 (ω-3) in die neutrale lipiede geproduseer het. *Saccharomycopsis vini* het die laagste persentasie 16:0 in hierdie fraksie geproduseer. *Saccharomycopsis fibuligera* en *S. schoenii* het onderskeidelik die

hoogste persentasies 16:0 en 18:2 in die glikolipiedfraksie geproduseer. *Saccharomycopsis javanensis* en *S. synnaedendra* het onderskeidelik die hoogste persentasies 18:1 en 16:1 in die fosfolipiedfraksie geproduseer. Alhoewel dit moontlik was om mb.v. hierdie fenotipiese eienskap tussen die meeste spesies te onderskei, kon hierdie vetsure nie gebruik word om te voorspel watter soorte 3-OH vetsure hierdie spesies kan produseer nie.

Saccharomycopsis fermentans (nuwe ongeïdentifiseerde 3-OH vetsuur), *S. malanga* (3-OH 16:0), *S. synnaedendra* (3-OH 16:0, 3-OH 17:0, 3-OH 18:0, 3-OH 18:1, 3-OH 19:0, 3-OH 19:1, 3-OH 20:0, 3-OH 22:0) en *S. vini* (3-OH 9:1, 3-OH 10:1) kon onderskei word m.b.v hierdie eienskap. Alhoewel, *S. capsularis* en *S. javanensis* albei 3-OH 9:1 geproduseer het, kon vetsure met 'n onewe getal koolstofatome wat kan dien as voorlopers egter nie in die neutrale, gliko- of fosfolipiede opgespoor word nie.

Key words: Bioprospecting, Fatty acids, Gas chromatography – mass spectrometry, 3-Hydroxy oxylipins, Immunofluorescence, Lipids, *Saccharomycopsis*, Taxonomy, Yeast

Sleutel woorde: Bioprospektering, Vetsure, Gaschromatografie – massa-spektrometrie, 3-Hidroksie oksilipiene, Immunofluoresensie, Lipiede, *Saccharomycopsis*, Taksonomie, Gis