Details on Project: Anti-fungal effect of unusual fatty acids

Focus Area

OTHER

Particulars of Applicant

Project Leader	Dr Carolina (CH) Pohl
Faculty	Natural and Agricultural Sciences
Department/Unit/ Centre	Microbial Biochemical and Food Biotechnology

Proposal Details

Title	**The effect of unsaturated C20 fatty acids, including non-methylene interrupted fatty acids on pathogenic fungi**
Description	Due to the unique chemical structure of non-methylene interrupted fatty acids (NMIFAs) such as sciadonic acid, they may be incorporated into mammalian phospholipids in a manner similar to and competing with methylene interrupted fatty acids, especially arachidonic acid (Tanaka et al., 2001; Berger et al., 2002). These NMIFAs can however not be metabolised to produce eicosanoids, which are important regulators of host immune responses (Berger & Jomard, 2001; Tanaka et al., 2001; Berger et al., 2002). This provides these fatty acids with anti-inflammatory properties. Several patents are registered exploiting this anti-inflammatory role of NMIFAs (Berger & Jomard, 2001). Since it is known that several pathogenic fungi utilise the arachidonic acid cascade or are capable of producing arachidonic acid metabolites either as a mechanism of virulence or as morphogenic factors (Noverr et al., 2002), it will be of interest to determine the effect of exogenous NMIFAs on the growth, metabolism and life cycle of fungi and yeasts. Of special interest to the focus area will be the influence of exogenous NMIFAs on potentially pathogenic yeasts and fungi such as Candida, Cryptococcus and Aspergillus. The possibility of using these fatty acids as novel antifungal agents in future will be assessed. The influence of these unusual fatty acids on the lipid metabolism of non-pathogenic fungi and yeasts, such as Dipodascopsis, Saccharomyces and Penicillium will also be of interest since new potentially biologically active oxylipins may be formed when these fungi are exposed to them.
Funding	Thuthuka-NRF

Details on Project: Lipid Biotechnology

Focus Area

Economic growth and international competitiveness

Particulars of Applicant

Project Leader	Prof Lodewyk (JLF) Kock
Faculty	Natural and Agricultural Sciences
Department/Unit/ Centre	Microbial Biochemical and Food Biotechnology

Proposal Details

Title	**Oxidised lipid research to benefit industry, science and society**
Description	Description and contributions to field of research Oxylipins are saturated and unsaturated oxidized fatty acids that are widely distributed in nature i.e. plants, animals and in some micro-organisms as constituents of various complex lipids or as free carboxylic acids. These compounds include the eicosanoids (e.g. prostaglandins, thromboxanes, leukotrienes, lipoxygenase products), many of which are pharmacologically potent compounds with important biological activities [1 - 4]. Also included are a large number of hydroxyoxylipins, which are formed by either lipoxygenase, dioxygenase or cytochrome P-450 mediated pathways which have been reported in fungi [5-10]. These compounds carry one or more hydroxy groups at carbon atoms 5,7,8,9,12,13,15 or 17 of the fatty acid molecule and are mostly formed from oleic or linoleic acid. In 1988, an extensive bioprospecting program was launched by Kock and co-workers from South Africa to determine if yeasts can produce acetylsalicylic acid (aspirin) sensitive oxylipins such as prostaglandins. The reason? These autacoids (chemically produced and very expensive) are administered to elicit several responses in humans e.g. labor induction [1, 11] - therefore a cheaper biotechnological source for these compounds will have obvious advantages. Using radio TLC and RIA, the presence of these compounds as well as other oxylipins could be demonstrated when the direct precursor arachidonic acid (AA) was fed [11]. Consequently, the practical application of this discovery was also included in several patents by Kock [12,13,14,15]. In one of his patents, applied for on 7 June 1990 [13], Kock suggested the use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) to combat fungal infections. Here, NSAID-sensitive oxylipins were regarded as the target site. Surprisingly it was discovered, using radio TLC in combination with H1 2DCOSY NMR, gaschromatography mass spectrometry (EI & FAB) as well as IR spectroscopy analyses, that a novel acetylsalicylic acid sensitive 3-hydroxy polyunsaturated oxylipin i.e. 3R-hydroxy 5Z, 8Z, 11Z, 14Z eicosatetraenoic acid (3R-HETE) is produced by the yeast Dipodascopsis uninucleata var. uninucleata [16]. This only happened when fed with AA, a precursor for prostaglandin formation in humans [1]. Later studies by both South African and UK groups show that this yeast is capable of producing a wide variety of novel 3-hydroxyoxylipins when fed with different precursors i.e. 3-hydroxy 20:3; 3-hydroxy 20:5; 3-hydroxy 14:2; 3-hydroxy 14:3 [17, 18]. Before this discovery, only the presence of saturated 3-hydroxyoxylipins, with no referral to function, was reported in yeast by various researchers [4, 19]. Later, the production of 3R-HETE was reported by Kock et al. [20] in D. tothii, a close relative of D. uninucleata based on rDNA comparisons [21]. The first evidence concerning the biological activity of 3R-HETE was presented by German and South African groups in the early 1990's. It was reported that this compound affects signal transduction processes in human neutrophils and tumour cells in multiple ways [22] thereby rendering a biotechnological value to this compound. Of course curiosity also centered on the functions of this compound in fungi especially yeast! In order to determine this, the affects of different low concentrations of acetylsalicylic acid on the life cycle of this yeast when cultivated in synchrony was studied [23]. It was found that the most susceptible part of the life cycle towards this cyclooxygenase inhibitor was the sexual stage i.e. the liberation of ascospores from the ascus. Coinciding with this, the production of 3R-HETE (found to occur mainly during sexual spore formation) was severely inhibited [24].

	In 1997 the life cycle of this yeast was mapped for the production of 3R-HETE making use of polyclonal antibodies against this compound. This was the start of a new phase in this field of research. For this purpose, the oxylipin was first synthesized by Bhatt from the USA for antibody preparation [25]. The synthetic strategy for the production of 3R- and 3S- HETE, involved a convergent approach coupling chiral aldehyde with Wittig salt: these were derived from 2-deoxy-D-ribose and AA, respectively [25]. Other novel synthetic routes were proposed in 2002 and 2004 by the Russian research group [26, 27]. Next, antibodies against chemically synthesised 3R-HETE were raised in rabbits and then characterised by determining its titer, sensitivity and specificity. It was found that the antibodies were specific for 3-hydroxyoxylipins in general (cross-reactions occurred with different 3-hydroxyoxylipins) which assisted in mapping 3-hydroxyoxylipins with different chain lengths and desaturation levels. Immunofluorescence microscopy of fungal cells at different stages of the life cycle indicated that these oxylipins are associated with the surface of aggregating sexual spores also known as ascospores [28]. Strikingly, according to transmission electronmicroscopy and oxylipin inhibition studies, 3-hydroxyoxylipin associated ascospore ornamentation i.e. nano-scale hooks were responsible for ordered ascospore liberation [29]. It was also reported that the formation of these hooks is inhibited by low concentrations (0.1 mM) acetylsalicylic acid [29]. After viewing many hours of video enhanced imaging displays [30] showing ascospore release as well as performing light- and electron microscopy, Kock and co-workers [29] concluded that surface ornamentation plays an important role in ascospore release and ordered reassembly outside the ascus of D. uninucleata. This is the first report elucidating the function of ascospore surface structures in fungi [29]. Oil "lubricated" ascospores (the oil phase later found by Smith et al. [31] using immunogold labeling to contain 3-hydroxyoxylipins) are at first perfectly interlocked through nano-scale surface hooks at the base of the ascus. As turgor pressure starts to increase, these spores disassemble (hooks unlock) as they are forced along a complex runway that tapers down as it reaches the ascus tip. At this stage only one spore at a time is forcibly propelled from the ascus through the tip just to be trapped in an elegant orderly manner outside the ascus i.e. packet of spores (spores in unlock position) by a 3-hydroxyoxylipin containing matrix that are released together with the spores to eventually form an interspore matrix attaching to the hooks. It was also reported that surface ornamentation is not limited to ascospores. Using relevant electronmicroscopy including immunogold labeling and immunofluorescence microscopy the Kock group found that flocculating vegetative cells of Saccharomyces cerevisiae contain interesting oxylipin associated "sticky" ornamentations that play a role during flocculation [30, 32]. These oxylipins were found to be 3-OH 8:0 and 3-OH 10:0 which form part of "sticky" probably lectin associated protuberances that migrate through the cell walls and attach to adjacent cells. Next, the in situ occurrence and localisation of 3-hydroxyoxylipins in yeast and other fungi were mapped. Surprisingly, 3-hydroxyoxylipins, many of which the complete chemical structures are only partially uncovered, were found to be produced by various yeasts and mucoralean fungi – in all cases these compounds were associated with the surfaces of aggregating vegetative- and sexual spores implicating an adhesive role probably through entropic based hydrophobic forces. As found previously in D. uninucleata, fluorescence was associated with the closely associated sexual cells (ascospores) of many lipomycetaceous species tested i.e. Lipomyces doorenjongii, L kockii, L. kononenkoae, L. starkeyi, L. yamadae, L. yarrowii, Smithiozyma japonica and Zygozyma oligophaga. However, in contrast to D. uninucleata, 3-hydroxyoxylipins (i.e. 3R-HETE) accumulated on the ascus tip of the closely related D. tothii and less was observed between the aggregating ascospores as observed by immunofluorescence microscopy [33]. The adhesive role of 3-hydroxyoxylipins (i.e. 3-OH 16:0) was further illustrated in the yeast Saccharomycopsis malanga where these compounds were observed by electron microscopy as micellar threads linking aggregating vegetative cells [34,35]. These oxylipins were also reported in the pathogenic yeast Candida albicans. Novel 3-hydroxyoxylipins were observed on the surface of the filamentous structure and played a role in morphogenesis and possibly pathogenicity of this yeast [36, 37, 38]. Strikingly in 2001, Noverr and co-workers from the USA confirmed the discovery by Kock's laboratory [11] when they demonstrated that the pathogenic yeasts Cryptococcus neoformans and Candida albicans produce immunomodulatory prostaglandins [39, 40]. A review by Noverr and co-workers then followed in 2003 acknowledging the Kock-discovery and the possible role of oxylipins as virulence factors [41]. In 2004 Alem and Douglas from the UK [42] demonstrated that biofilms formed by Candida albicans can be inhibited as much as 95% by aspirin. Strikingly, when prostaglandin E2 was added together with aspirin, the inhibitory effect of aspirin

was abolished! They concluded that aspirin possesses potent antibiofilm activity in vitro and could be useful in combined therapy with conventional antifungal agents in the management of biofilm-associated Candida infections. Strikingly, in 2005 the same authors independently from the Kock-group, confirmed the Kock-discovery and suggested that prostaglandin production could be a virulence factor in yeast biofilm-associated infection [43].

Recently, Kock and co-workers [44], proposed the likely primary function of oxylipin-lubricated yeast meiospore shape and nano-scale ornamentations, in water-driven movement. Here, aspirin-sensitive 3-hydroxyoxylipins act as prehistoric lubricants involved in the release mechanics of these spores from enclosed asci, probably for dispersal purposes. This interpretation may find application in nano-, aero- and hydro-technologies. In the same year plant pathologists from the University of Wisconsin (USA) proposed that oxylipins may play a role as regulators in sexual and asexual spore formation in Aspergillus nidulans [45].

Conclusions

The study on prostaglandins and 3-hydroxyoxylipins so far implicates a wide distribution of a large variety of these compounds in the fungal domain. As this mystery unfolds, it will be of interest to determine the biological function as well as taxonomic value of these oxylipins in these organisms. Especially the production of novel 3-hydroxyoxylipins e.g. 3-OH 20:4 (only found so far in Dipodascopsis), 3-OH 14:2 (only so far in Dipodascopsis and Mucor genevensis), 3-OH 10:0, 3-OH 8:0 (in Saccharomyces cerevisiae) and others seems to have taxonomic significance and literature suggests that these compounds may have the potential to be used as markers to identify fungi. The value of this phenotypic characteristic to be used as a rapid identification tool in biotechnologically important fungi therefore warrants further research. Such a study is not only of taxonomic importance but can also assist in the quality control programs used in biotechnological processes.

It can be concluded from this review that prostaglandins and 3-hydroxyoxylipins are apparently associated with yeast structures that tend to aggregate. Consequently, a kind of adhesion function may be attributed to these compounds. Whether this occurs through signaling pathways and/or entropic based hydrophilic forces or hydrogen bonds is yet to be verified [34, 46, 47]. Moreover, the 3-hydroxyoxylipin structure may be of importance in adhesion. For instance, in S. malanga, 3-hydroxyoxylipin-threads are present as micellar–like units characterized by a hydrophilic outer layer and a more hydrophobic inner part [34]. This may be ascribed to a larger polar head of the 3-OH 16:0 (forming the outer part of the micelle) compared to the hydrocarbon chain, hence this 3 hydroxyoxylipin are more likely to form thread-like micelles [46] which in turn may attach to the hydrophilic cell walls of the vegetative cells. This aspect however needs more clarification concerning other types of 3-hydroxyoxylipins. Recently a novel 3-hydroxyoxylipin, 3,18-dihydroxy-5,8,11,14-eicosatetraenoic acid was identified by the German group in Candida albicans a pathogen in vulvovaginal candidiasis [36, 37, 38]. These researchers concluded that the administration of acetylsalicylic acid (aspirin) should be beneficial in the treatment of this disease by dual ways: (i) by inhibiting the 3-hydroxyoxylipin formation - mainly associated with the hyphal phase, and (ii) by inhibiting prostaglandin E2 formation in the infected host tissue. Who knows what secrets are locked up in the functional role of other 3-hydroxyoxylipins? What happens if these oxylipins are added to fungal and mammalian cells? The role of these oxylipins may not only be of importance in medical applications but may be of importance in the sedimentation of cells in biotechnological processes such as brewing where cells are at present mainly removed from culture by expensive centrifugation. Ways to control yeast flocculation in the brewing process has been the subject of many studies the past 30 years. Strikingly, in 2005 Strauss et al. [48] demonstrated that 3-OH oxylipins are involved in yeast flocculation and that aspirin partially inhibits this process. 3-Hydroxyoxylipins have also been detected in various other yeasts such as Dipodascus [49], Ascoidea africana [50] and Eremothecium coryli [51]. In 2005, the laboratory of Prof Nigam discovered 3-OH prostaglandins [52] mediated by Candida albicans probably upon infection.

In 2003, Kock discovered [44] that these oxylipins also serve as lubricants used by fungi to discharge spores for smart dispersal in micron-space. This new field of research, which may find application in nanotechnology, was introduced by Kock in 2006 [53] on invitation to the field of engineering. Will this discovery have the same impact as that experienced in the medical field [54]?

Inhibition studies so far on 3-hydroxyoxylipins have partially unlocked the secret concerning the function of surface structures in yeast. The impact of further studies in this field will be followed with interest. More references from the Kock group can be

obtained under the Publications Section.

SKILLS OBTAINED FROM ABOVE RESEARCH HAVE BEEN SUCCESSFULLY APPLIED TO THE EDIBLE OIL INDUSTRY OF SOUTH AFRICA I.E. TO TERMINATE MISREPRESENTATION AND OVERUSE PRACTICES (REFER TO COMMUNITY SERVICE SECTION).

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