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10. Production of bacterial oxylipins by *Pseudomonas aeruginosa* 42A2

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Abstract. Oxylipins are a family of natural compounds that are reported to perform a variety of biological functions. Besides the biological properties of such compounds, interest in hydroxy fatty acids is increasing, due to the industrial applications of these renewable compounds as a starting material for resins, emulsifiers, plastics or polyesters. Hydroxy fatty acids are used as thickeners in a new generation of emulsifiers and lubricants, to reach new levels of performance. When grown in submerged culture with oleic or linoleic acid, Pseudomonas aeruginosa 42A2 produced several oxylipins. In this study, oxylipin production and its applications are examined.

Introduction

The main sources of hydroxy fatty acids (HFAs) are plants and a great variety of seed oils, particularly castor oil. In fact, HFAs are ubiquitous compounds in nature, as they are found in animals, fungi and bacteria. Classical

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oleochemistry occurs mainly at the ester functionality of the triglycerides. Whereas most native oils contain unsaturated fatty acids, as Biermann and collaborators stated, only a few reactions across the double bond of unsaturated FA are currently applied [1]. Numerous synthetic problems remain unsolved, and solutions must be found. As described in the literature, a great number of prokaryotes may be used for biotransformation processes. Microorganisms contain a wide range of enzymes for biotransformation, e.g. lipases, lipoxygenases, oxidases, epoxidases, P450 monooxigenases, that have enormous potential to develop new biotechnological processes with unsaturated fatty acids, making it possible to obtain a new brand of products with unexplored properties. *Candida* sp. or *Bacillus* sp. are reported to produce diacids (ω-unsaturated fatty acids), which are important compounds for polyester or polyamide synthesis [1], and epoxy fatty acids, which are used as PVC stabilizers or as starting materials to produce polyether polyols and are also found in reactions produced by the *Gaeumannomyces graminis* fungus [2]. The oxidation of unsaturated fatty acids is now focused on epoxidation, hydroxylation and double bond cleavage, to be used in fine chemistry and for further polymerization [1].

Microbial transformation is a powerful strategy to develop new lipid products. Lipases are the most common enzymes used in oleochemical processes. These enzymes do not require cofactors for catalysis, and show stereo-, regio- and chemoselectivity, high activity and stability. Most of them, e.g. those from *Pseudomonas cepacia*, *Candida antarctica*, *Rhizomucor miehei* or *Rhizopus delemar*, are commercially available. Recently, an increasing number of microorganisms producing HFAs have been reported as an efficient tool to convert inexpensive unsaturated fatty acids into value-added compounds [3]. Oxidative enzymes belonging to the P450 superfamily have been involved in α-hydroxylation or in the fatty acid synthase and β-hydroxylation systems in the case of β-HFAs; cytochrome P450 monooxygenases from different microorganisms, e.g. *Bacillus* or *Pseudomonas*, can produce ω-HFAs; and bacterial monooxygenases have been heterologously expressed [3]. Mid-position hydroxylation on saturated HFAs has been reported by Wallent [4] and Volkov [5]. Unsaturated HFAs have also been reported; the resulting product might be mono- or poly-HFAs [3]. The enzymes involved in these reactions are lipoxygenases and diol synthases [6, 7].

In nature, oxylipins have different functions. They act as signalling molecules and biological mediators, and they facilitate the plasticity of cell

membranes, the mobilization of lipids [8] and the generation of HFAs that are involved in cellular communication and the antibiosis effect. Although in minute amounts, polyesters of HFAs have been detected in oily seeds and in animal organisms, where they play the role of natural lubricants [9, 10].

The antimicrobial activity of such compounds and the functionality of the HFAs have attracted attention, leading to the generation of new polyesters, called estolides, which form a new class of green emulsifiers and lubricants. In this study, the production of hydroxy fatty acid derived from oleic acid (OA) and linoleic acid (LA) is described, as well as the applications of these compounds.

1. Microbial conversion of oleic acid

Since the first report of the microbial conversion of OA to 10-hydroxystearic acid by a *Pseudomonas* [4], scarce information has been published about the hydroxylation of OA whilst maintaining the unsaturated alkyl-chain. Heinz and co-workers reported that *Torulopsis* sp. produced 17-hydroxyoleic acid [11], and Soda and collaborators described the conversion of OA into ricinoleic acid (RA) by *Bacillus pumilus* [12]. Other uncommon HFAs have been described, such as 15-, 16-, 17-hydroxy-9*Z*-octadecenoic acid by *B. pumilus* [13] or 3-hydroxy-9*Z*-octadecenoic acid by *Alcaligenes* sp. 5-18 [14].

Although 7*S*,10*S*-dihydroxy-8*E*-octadecenoic acid ((7*S*,10*S*)-DiHOME) produced by *Pseudomonas aeruginosa* 42A2 was the first dihydroxylated derivative from OA to be described [15], the accumulation of HFAs has also been reported for several *P. aeruginosa* strains [16]. *Pseudomonas* 42A2 NCIMB 40045 is a gram negative bacterium with a versatile metabolism, which is the reason for its ubiquitous characteristic of being associated with other free-living soil microorganisms. It has the ability to transform long-chain unsaturated fatty acids into oxylipins.

1.1. Batch production

P. aeruginosa 42A2 accumulates several oxidized fatty acids, namely 10*S*-hydroperoxy-8*E*-octadecenoic acid ((10*S*)-HPOME), 10*S*-hydroxy-8*E*-octadecenoic acid ((10*S*)-HOME) and 7*S*,10*S*-dihydroxy-8*E*-octadecenoic acid (7S,10S- DiHOME), when it is incubated with OA as a single substrate in a minimal mineral medium (Fig. 1).

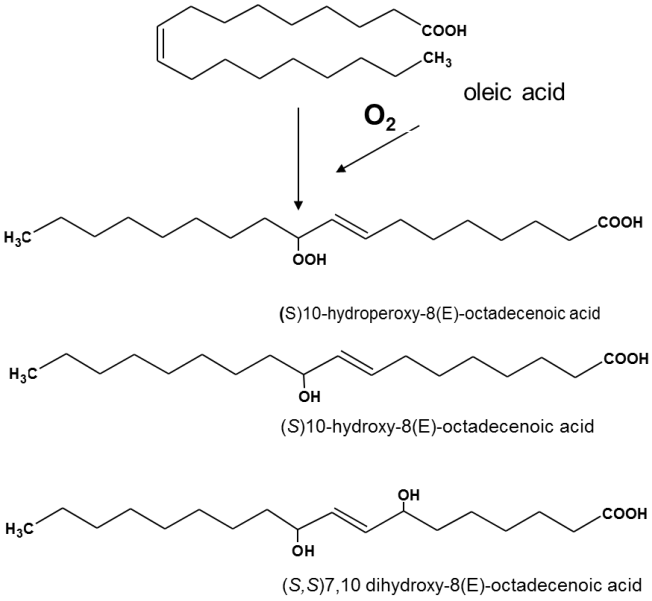

Figure 1. Products obtained from the conversion of OA by *P. aeruginosa* 42A2.

Although (10*S*)-HOME was first identified as the precursor of (7*S*,10*S*)-DiHOME [17], Martinez and co-workers suggest that the precursor of (7*S*,10*S*)-DiHOME is (10*S*)-HPOME, and that (10*S*)-HOME is a by-product obtained from the spontaneous reduction of (10*S*)-HPOME [6]. Subsequently, the two enzymes involved in the reaction were identified and the genomic structure of the diol synthase operon was elucidated. Thus, OA is transformed into (10*S*)-HPOME in a first enzymatic reaction, in order to produce (10*S*)-HOME spontaneously, and (7*S*,10*S*)-DiHOME is generated in a second enzymatic reaction. All of this enzymatic system is located in the periplasm of *P. aeruginosa* 42A2 [18].

Most microbial processes require air for cell growth. In some cases, foam accumulates during production, and can greatly disturb the process. To overcome foam formation in the production of (7*S*,10*S*)-DiHOME, which has surface activity properties, an innovative non-dispersive aeration system was designed. It is based on Higbie's penetration theory applied to a wetted-wall column. Hence, an innovative bioreactor was created for a foaming biotransformation [19].

The biotransformation kinetics were deduced by varying the substrate concentration from 10 to 20 $g \cdot L^{-1}$ to obtain different experimental data sets. The typical time course of the OA transformation (Fig. 2) indicated that (10*S*)-HPOME accumulation started soon after inoculation until the end of the exponential phase of cell growth. Most (7*S*,10*S*)-DiHOME and (10*S*)-HOME accumulation occurs during the stationary phase until the end of the process (30 h), which confirms the role of these compounds as secondary metabolites. A high conversion yield ($Y_{P/S}$) 0.9 was achieved and a cell yield ($Y_{P/X}$) of 3.2 was calculated. 10*S*-HPHOME was not detected at the end of the culture.

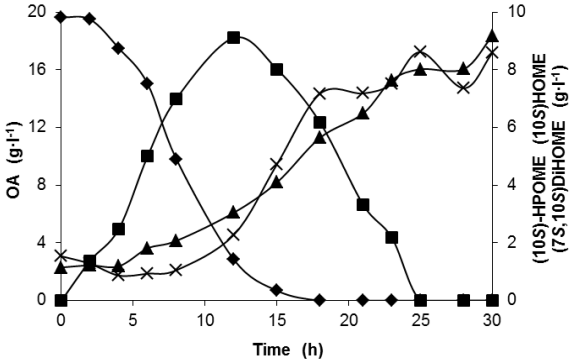

Figure 2. Kinetics of OA (♦) conversion by *P. aeruginosa* 42A2 (OA of 20 $g \cdot L^{-1}$) into (10*S*)-HPOME (■), (10*S*)-HOME (▲) and (7*S*,10*S*)-DiHOME (x).

As shown in Table 1, the volumetric productivity (P_V) that was calculated was substrate dependent. (10*S*)-HOME followed the same pattern as (10*S*)-HPOME, whereas the accumulation of (7*S*,10*S*)-DiHOME depended on the reaction of (10*S*)-HPOME with the hydroperoxy-diol synthase enzyme. Therefore, the production rate was proportional to the amount of (10*S*)-HPOME [18, 19].

Table 1. Effect of oleic acid concentration on volumetric productivity.

OA ($g \cdot L^{-1}$)	(10*S*)-HPOME ($g \cdot L^{-1} \cdot h^{-1}$)	(10*S*)-HOME ($g \cdot L^{-1} \cdot h^{-1}$)	(7*S*,10*S*)-DiHOME ($g \cdot L^{-1} \cdot h^{-1}$)
10	0.41	0.15	0.34
15	0.47	0.21	0.25
20	0.75	0.29	0.31

As expected, the experimental data fitted the mathematical model proposed by Monod, which corresponds to first-order reactions occurring during the biotransformation of OA into these oxidized compounds [19].

Due to the nature and functionality of the (10*S*)-HOME and (7*S*,10*S*)-DiHOME accumulated in the cultures, they might easily be further modified to form a new generation of polyesters named estolides (ESTs). ESTs are a class of polymeric secondary esters derived from addition across an unsaturated bond or with HFAs by a carboxyl moiety of another fatty acyl group.

2. Estolide production

Although in a minuscule amount, ESTs are present in nature in the epicuticular wax of some species of *Juniperus*, *Pinus* or *Coniferae*, and in the oil seeds of certain plants [20] or fungi [21]. ESTs are also found in the animal kingdom, in wool wax, beeswax [22], and in human meibomian gland secretion [23]. Furthermore, ESTs have been found in cultures of *Pseudomonas aeruginosa* 42A2 when cultivated with OA as substrate [24].

Yamaguchi and collaborators reported for the first time the esterification of castor oil in a two-step enzymatic reaction to obtain ESTs with a high yield [25]. This led to a new generation of environmentally friendly emulsifiers for a wide range of applications in the food industry. Particularly important are the RA ESTs, which are used as intermediates in the enzymatic production of polyglycerol polyricinoleate (E-476), as reported by Bodalo and co-workers [26, 27].

Two types of ESTs were synthesized from (10*S*)-HOME and (7*S*,10*S*)-DiHOME, using the thermostable commercial lipases Novozym 435 (lipase B from *Candida antarctica*), Lipozyme RM IM (*Rhizomucor miehei* lipase), and Lipozyme TL IM (*Thermomyces lanuginosus* lipase). Enzymatic reactions were carried out at 80 ºC under vacuum conditions to reduce the viscosity of HFAs monomers [28]. As shown in Fig. 3, the time course of the reaction using Novozym 435 (N435) offered the best performance with both HFAs. However, Lipozyme RM IM (L RM IM) behaved in a similar way to N435 when the substrate was (10*S*)-HOME. In contrast, Lipozyme TL IM (L TL IM) registered yields that were slightly lower than N435 in the polymerization of (7*S*,10*S*)-DiHOME.

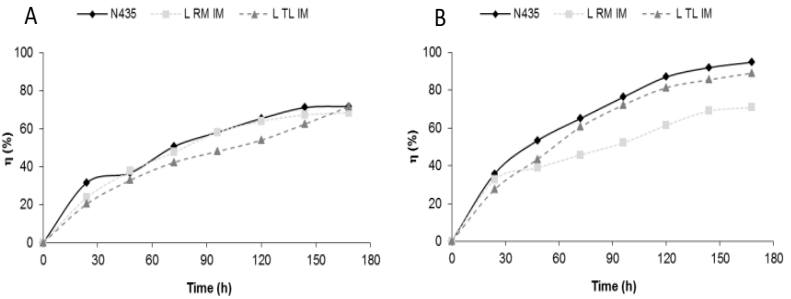

Figure 3. EST enzymatic production with 12% (w/w) of the corresponding lipase at 80 ºC for 168 h under vacuum conditions. A: (10*S*)-HOME; B: (7*S*,10*S*)-DiHOME.

Borgdorf and Warwel classified lipases according to their ratio competitive factor (RCF) [29]. This factor describes the selectivity of one single lipase toward two substrates with the same leaving group, and to two acyl groups.

Table 2. Reaction yield (%) in estolide formation from the oxylipin produced by *P. aeruginosa* 42A2 from oleic acid.

Lipase	RCF (%)	(10*S*)-HOME (%)	(7*S*,10*S*)-DiHOME (%)
Novozym 435	0.7	71.7	94.7
Lipozyme RM IM	1.3	68.4	70.8
Lipozyme TL IM	_	71.6	88.9

Although the lipase RCF should be taken into account, no relationship with the reaction yield was observed (Table 2). Bodalo and co-workers found that *sn*-1,3 selective lipases are unable to attack secondary alcohols [30]. However, the *sn*-1,3 specific lipases Lipozyme RM IM and Lipozyme TL IM had significant reaction yields with both substrates. The non-specific lipase Novozym 435 was the best catalyst with the highest reaction yields (71.7% and 94.7%), which indicates the importance of the nature of the lipase and the substrate.

There are no reports in the literature on EST synthesis from *trans*-HFA monomers. However, Aguieiras and partners used these enzymes in the production of mono-ESTs from OA and methyl ricinoleate, and achieved a

reaction yield of 33% after 48 h at 80 ºC using 6% enzyme (w/w) [31]. This reaction was also studied by Horchani and collaborators using the immobilized lipase of *Staphylococcus xylosus*. A 65% reaction yield was achieved after 55 h at 55 ºC [32].

Liquid chromatography coupled to MS has been found to be a time-consuming technique, especially the optimization of mass spectrometer potentials to obtain a low amount of fragmentation in the mass spectra of large molecules. In contrast, MALDI-TOF analyses carried out with a proper matrix and stabilization cation, e.g. Li^{+7} or Na^{+23}, is a fast technique with low fragment mass spectra, which means that the method could elucidate the number of oligomers synthesized. Finally, nuclear magnetic resonance (NMR) is a good complementary structural technique to MALDI-TOF, which provides characteristic chemical shifts to identify *trans*-ESTs. *trans*-EST structural characterization was carried out by MALDI-TOF mass spectrometry and NMR. MALDI-TOF analyses were carried out using a 2,5-dihydroxybenzoic acid (DBH) matrix saturated with acetonitrile [33]. ESTs from (10*S*)-HOME (M) are presented in Fig. 4A.

Some ions stand out from the rest, which means that the oligomers that are produced can be identified easier and faster. The following ions were detected *m/z* 601.5, $[2M\text{-}H_2O+Na^{23}]^+$; 881.7, $[3M\text{-}2H_2O+Na^{23}]^+$; 1162.0, $[4M\text{-}3H_2O+Na^{23}]^+$ and 1442.2, $[5M\text{-}4H_2O+Na^{23}]^+$. Hence, up to oligomers of five monomeric units of (10*S*)-HOME were synthesized. However, more ions were detected when (7*S*,10*S*)-DiHOME (M') was the substrate used (Fig. 4B): *m/z* 633.5, $[2M'\text{-}H_2O+Na^{23}]^+$; 929.7, $[3M'\text{-}2H_2O+Na^{23}]^+$; 1226.0, $[4M'\text{-}3H_2O+Na^{23}]^+$; 1522.3, $[5M'\text{-}4H_2O+Na^{23}]^+$; 1819.5, $[6M'\text{-}5H_2O+Na^{23}]^+$; 2115.7, $[7M'\text{-}6H_2O+Na^{23}]^+$; and in the enlarged region of the spectra the following ions were observed 2412.0, $[8M'\text{-}7H_2O+Na^{23}]^+$; and, 2709.2, $[9M'\text{-}8H_2O+Na^{23}]^+$. In this situation, up to oligomers of nine monomeric units of (7*S*,10*S*)-DiHOME were produced, as expected from the reaction yields presented in Table 2.

Furthermore, NMR spectroscopy was used to confirm the results observed in the MALDI-TOF analysis. Certain chemical shifts are decisive in order to identify *trans*-ESTs. In ^1H NMR spectra, these are the signals 2.28 ppm and 5.20 ppm, which correspond to the methylene protons (position 2) and to the methine proton (position 10) that are both adjacent to an ester group, respectively.

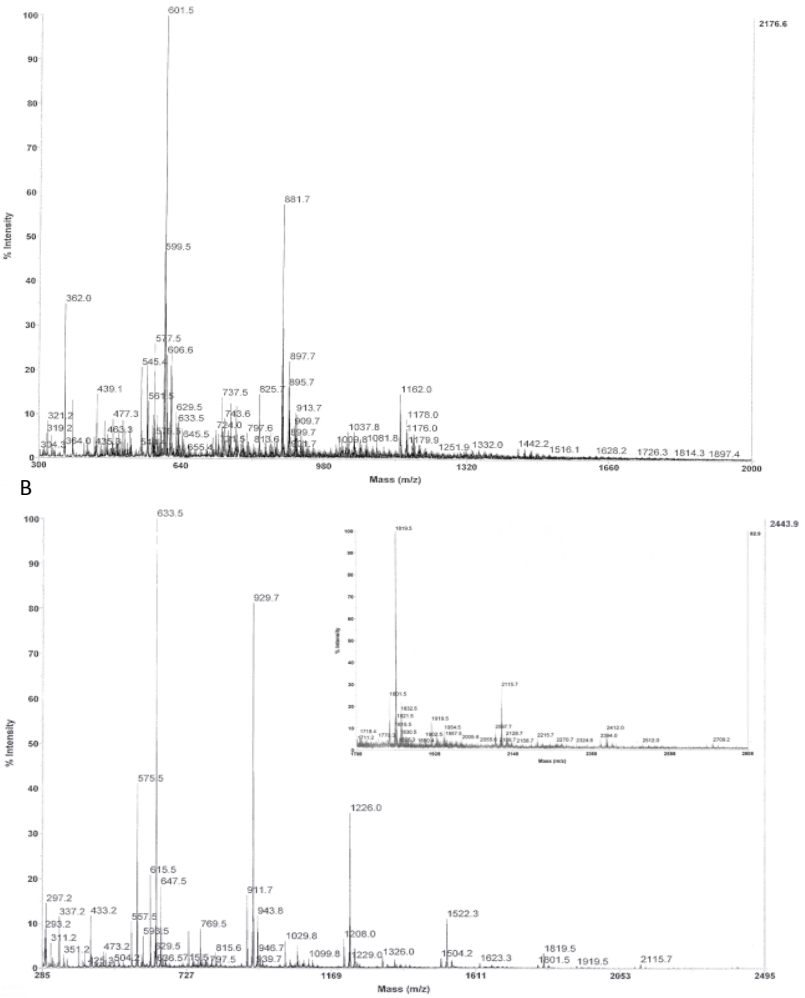

Figure 4. MALDI-TOF mass spectra with a DHB matrix saturated in acetonitrile. ESTs produced with Novozym 435 at 80 ºC under vacuum conditions for 168 h. A: (10*S*)-HOME ESTs; B: (7*S*,10*S*)-DiHOME ESTs.

An average number of monomers present in a sample could be calculated by integrating the methylene proton signal of 2.28 ppm. For ^{13}C

NMR spectra the determining signals are 74.50 ppm (position 10) and 173.17 ppm (position 1), which represent the presence of a tertiary carbon and an ester carbonyl, respectively. Fig. 5 represents the structure of ESTs of three monomeric units.

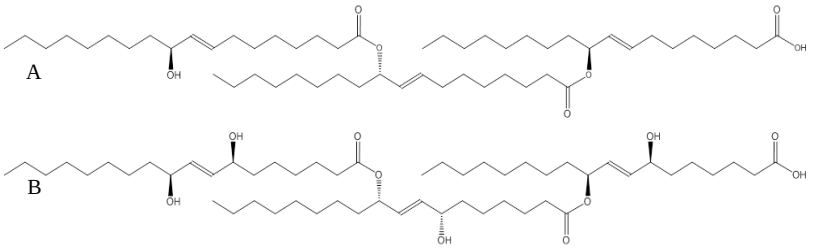

Figure 5. ESTs derived from (10*S*)-HOME (A) and (7*S*,10*S*)-DiHOME (B).

Finally, some physicochemical properties were determined for the produced ESTs: the Arrhenius relationship between viscosity and temperature (A and E_a), the glass transition temperature (T_g), and decomposition enthalpies (ΔHi).

According to mass spectrometry analysis, the ESTs analysed were considered as oligomers and not strictly polymers, due to their low polymerization degree. Such simple molecules were determined as Newtonian fluids, because their viscosity remained constant over different shear rates. A dynamic or absolute viscosity (η) determination was run over three different temperatures (20, 40 and 60 ºC) when possible, to calculate the pre-exponential factor (A) and activation energy (E_a) of the Arrhenius-type relationship [34]. This equation describes the dependence of viscosity on temperature, where A can be considered as the infinite-temperature viscosity value and E_a indicates the sensitivity of a substance to temperature [35].

$$\eta = A \cdot \exp\left(-{}^{E_a}/_{R \cdot T}\right)$$

Physicochemical analyses results are listed in Table 3. The parameters include A and E_a, density (ρ), kinematic viscosity at 40 ºC, reaction yield (η) and oligomer number (ON), which is the maximum number of oligomers synthesized.

Table 3. Viscosity and characterization parameters from ESTs.

Sample	$A \cdot 10^{-10}$ (Pa·s)	E_a (kJ·mol^{-1})	ρ (g·mL^{-1})	ν (cSt)	η (%)	ON (–)
(10*S*)-HOME	9.47	49.9	0.96	205	–	1
(7*S*,10*S*)-DiHOME	n.d.	n.d.	0.99	n.d.	–	1
(10*S*)-HOME N435	3.48	55.1	0.97	608	71.7	6
(10*S*)-HOME RM IM	5.79	53.6	0.97	495	68.4	6
(10*S*)-HOME TL IM	24.2	49.3	0.97	402	71.6	6
(7*S*,10*S*)-DiHOME N435	3.62	59.1	1.00	2510	94.7	9
(7*S*,10*S*)-DiHOME RM IM	0.14	67.7	1.02	3235	70.8	9
(7*S*,10*S*)-DiHOME TL IM	1.06	62.7	1.00	3000	88.9	10

As can be seen, the viscosity parameters for the (10*S*)-HOME ESTs are lower than those for (7*S*,10*S*)-DiHOME ESTs. The same is true for ON values. (7*S*,10*S*)-DiHOME ESTs were produced in higher yields; thus, their ON values were higher. In addition, the melting point of (7*S*,10*S*)-DiHOME was 56 ºC, due to the hydrogen bond interaction of the two hydroxyl groups. Hence, (7*S*,10*S*)-DiHOME EST viscosity parameters had higher values.

Table 4. EST calorimetric parameters.

Sample	T_g (ºC)	T_{onset1} (ºC)	ΔH_1 (J·g^{-1})	T_{onset2} (ºC)	ΔH_2 (J·g^{-1})	T_{onset3} (ºC)	ΔH_3 (J·g^{-1})	Residue (%)
(10*S*)-HOME	-60	119	-7.08	196	47.33	315	-161.3	0.35
(7*S*,10*S*)-DiHOME	-49	185	37.79	277	-56.16	366	147.3	1.23
(10*S*)-HOME N435	-63	–	–	213	33.97	323	-174.1	2.27
(10*S*)-HOME RM IM	-64	–	–	217	35.73	322	-174.0	1.57
(10*S*)-HOME TL IM	-66	–	–	218	27.64	338	-252.9	1.73
(7*S*,10*S*)-DiHOME N435	-50	187	34.37	295	-30.18	362	-118.9	1.37
(7*S*,10*S*)-DiHOME RM IM	-49	200	28.22	302	-36.23	370	-105.5	1.12
(7*S*,10*S*)-DiHOME TL IM	-50	201	18.97	300	-37.07	364	-133.5	2.74

Finally, thermal analysis, DSC (differential scanning calorimetry) and TGA (thermal gravimetric analysis) were used to characterize the new polyesters. Thermal parameters are listed in Table 4. T_g could be calculated for synthesized polyesters and even for both HFAs, indicating that these compounds lacked a crystal structure. EST T_g values were practically the

same as their corresponding substrates, which confirmed the simplicity of these molecules. Furthermore, onset temperatures and decomposition enthalpy values were maintained. These two points suggest that the oligomers produced had a larger quantity of dimers and trimers than higher polymerized oligomers.

3. Microbial conversion of linoleic acid

Due to the limited natural HFAs supply, research has focused on engineering castor oil production and developing new engineered microorganisms for RA production [36] and new processes for the microbial transformation of unsaturated fatty acids into HFAs [3, 37, 38]. The occurrence of lipoxygenase [39] and diol synthase activity [6, 18] in *P. aeruginosa* reinforces the versatile metabolic activity of this bacteria to produce diverse polyol oxylipins. When cultivated in submerged culture with linoleic acid as a substrate, *P. aeruginosa* 42A2 accumulated HFAs in the culture, with different degrees of hydroxylation [40].

Several oxylipins have been characterized from LA acid to date, due to the activity of bacterial lipoxygenases. Oxidized LA derivatives are hydroperoxides (9*S*)- and (13*S*)-HPODE (9*S*-hydroperoxide-10*E*,12*Z*-octadecadienoic and 13*S*-hydroperoxide-9*Z*,11*E*-octadecadienoic acids, respectively). These hydroperoxides can spontaneously transform into their corresponding hydroxides ((9*S*)-/(13*S*)-HODE), epoxyalcohols or even keto fatty acids; these last two by the action of hematin [41]. Recently, 10-hydroxy-12,15(*Z*,*Z*)-octadecenoic acid produced by a rec-enzyme from *Stenotrophomonas maltophilia* was characterized [42].

Scarce information has been reported on the bacterial production of dihydroxyl fatty acid. Besides 9,10-hydroxystearic acid, only (7*S*,10*S*)-DiHOME [15, 16] and 9,12-dihydroxy-10*E*-eicosenoic acid have been described [43]. Several trihydroxy-fatty acids have been reported so far from the bacterial transformation of RA into 7,10,12-trihydroxy-8*E*-octadecenoic acid [44] and from LA 9,10,13-trihydroxy-11*E*-octadecenoic ((9,10,13)-TriHOME) and 9,12,13-trihydroxy-10*E*-octadecenoic ((9,12,13)-TriHOME) [40]. A unique strain of *Bacillus* has been reported to accumulate 12,13,17-trihydroxy-9*Z*-octadecenoic acid [45].

When a complex substrate of fatty acids (60% LA, 40% OA, palmitic, stearic and linolenic acid) was supplied into the culture of *P. aeruginosa* 42A2, a mixture of compounds was found. The organic extract from the culture media contained several oxylipins (Table 5). Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used to allow the

direct analysis of products and combine the resolution of LC with the detection specificity of MS/MS. This is a direct, fast method for detecting and resolving the structure of the products accumulated in the culture without the formation of derivatives.

Table 5. Fragment ions detected in the organic extract.

Product	Fragment ions (*m/z*)
(10*S*)-HOME	297 $[M-H]^-$; 279 $[M-H-H_2O]^-$; 154.9 indicating –OH at C10
(9*S*)-HODE	295 $[M-H]^-$; 277 $[M-H-H_2O]^-$; 171 indicating –OH at C9
(13*S*)-HODE	295 $[M-H]^-$; 277 $[M-H-H_2O]^-$; 195 indicating –OH at C13
(9,10,13)-TriHOME	329 $[M-H]^-$; 311.2 $[M-H-H_2O]^-$; 293 $[M-H-2H_2O]^-$; 127 indicating –OH at C9 and C10
(9,12,13)-TriHOME	329 $[M-H]^-$; 311.2 $[M-H-H_2O]^-$; 293 $[M-H-2H_2O]^-$; 129 indicating –OH at C12 and C13

In this case, isomers (9,10,13)-TriHOME and (9,12,13)-TriHOME are present in the ratio 3:1, which differs from that reported by Kim and partners (1:1) from the *P. aeruginosa* strain PR3 [46]. Due to the difficulty of separating these isomers, a multiple reaction monitoring (MRM) analysis was undertaken, in which the acquisition mode was based on the injection of the sample containing the ion to be studied, *m/z* 329, into the first quadrupole (Q1), fragmentation in the collision cell (Q2), and scanning of the product ions in the third quadrupole (Q3). The result is an MS/MS spectrum free of interferences where the most appropriate product ions are selected in the third quadrupole (Q3), *m/z* 127 and 129, to perform relative quantification and confirmation. Thus, it was seen (Fig. 6) that for ((9,10,13)-TriHOME) the characteristic transition was *m/z* 329–127, and for the other isomer, ((9,12,13)- TriHOME), the characteristic transition was *m/z* 329–129.

TriHOME compounds belong to a class of oxylipins with antifungal activity similar to those isolated from a variety of rice plant (9,12,13-trihydroxy-10*E*,15-octadecadienoic and 9,12,13-trihydroxy-10*E*-octadecanoic acids) suffering from black rot disease [47, 48] and involved in the defensive mechanism of plants [49, 50]. The presence of lipoxygenases in environmental bacteria [39] and diol synthase enzymes [18] suggests the involvement of the microbiota in the interaction with eukaryote organisms.

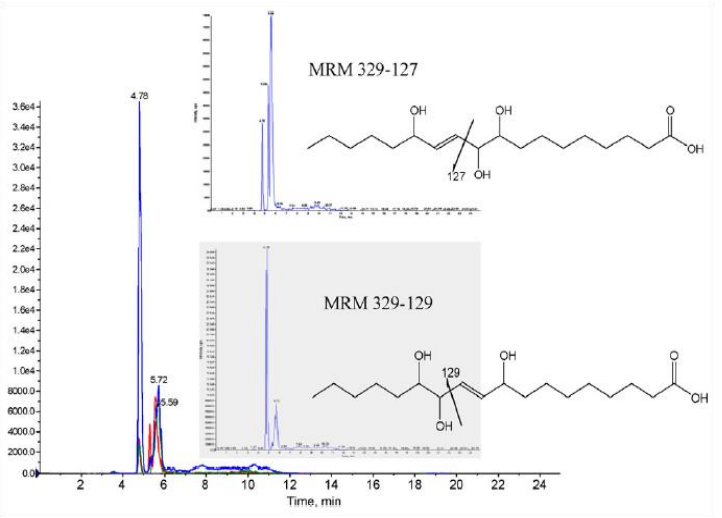

Figure 6. MRM analysis of trihydroxylated isomers derived from LA.

Although the mechanism of action of oxylipins is not yet understood, it has been suggested that the inhibition of growth may be due to the chemical or physical properties of oxylipins, rather than the interactions with specific cellular targets [50]. The oxylipins derived from OA, (10*S*)-HOME and (7*S*,10*S*)-DiHOME, could inhibit the growth of phytopathogenic fungal strains such as *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Drechslera teres* and produce an antibacterial effect against *Escherichia coli* and *Micrococcus luteus* (MIC 64 $\mu g \cdot mL^{-1}$); *Staphylococcus aureus* (MIC 32 and 8 $\mu g \cdot mL^{-1}$ (10*S*)-HOME and (7*S*,10*S*)-DiHOME, respectively) and *Bacillus subtilis* (MIC 32 and 128 $\mu g \cdot mL^{-1}$ (10*S*)-HOME and (7*S*,10*S*)-DiHOME, respectively) [51].

It was found that the oxylipins derived from LA cultures, those described in Table 5, were able to inhibit bacterial growth (Table 6), similarly to those described (9,10,13-TriHOME and 9,12,13-TriHOME) from the mildew fungus in barley, *Blumeria graminis*, which reduced infection by up to 42% when applied *in vivo* [52], or 10-hydroxy-8-octadecenoic and 10-hydroxy-8,12-octadecenoic acids, which were found in the stomata of timothy grass after being infected with *Epichloe typhina* [53].

Table 6. Minimal inhibitory concentration of the HFAs described in Table 5.

Microorganism	MIC ($mg \cdot ml^{-1}$)
Bacillus subtilis ATCC 6333	64
Enterococcus hirae ATCC 10541	32
Micrococcus luteus ATCC 9341	64
Arthroderma uncinatum ATCC 15082	32
Aspergillus brasiliensis ATCC 14404	140
Aspergillus repens IMI 016114	140
Macrophomina phaseolina IMI 48561	32
Penicillium chrysogenum ATCC 9480	140
Penicillium funiculosum CECT 2914	32
Tricophyton mentagrophytes ATCC 18748	64
Verticillium dahliae 49507/A	32

4. Conclusion

The transformation of unsaturated fatty acids by *P. aeruginosa* 42A2 produced several oxylipins with different degrees of hydroxylation. Further polymerization of oxylipins obtained from OA renders a new family of ESTs with properties of Newtonian fluids similar to natural lubricants. New oxylipins were produced from LA with biological properties against phytopathogenic fungal strains.

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