Review Article

New bioactive fatty acids

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Many oxygenated fatty acids are bioactive compounds. *Nocardia cholesterolicum* and *Flavobacterium* DS5 convert oleic acid to 10 hydroxy stearic acid and linoleic acid to 10-hydroxy-12(Z)-octadecanoic acid. *Pseudomonas aeruginosa* PR3 converts oleic acid to the new compounds, 7,10-dihydroxy-8(E)-octadecenoic acid (DOD) through 10-hydroxy-8-octadecenoic acid, and racinoleic acid to 7,10,12-trihydroxy-8-octadecenoic acid. DOD showed antibacterial activity including against food-borne pathogens. *Bacillus megaterium* ALA2 converted n-6 and n-3 PUFAs to many new oxygenated fatty acids. For example: linoleic acid was converted to12,13-epoxy-9-octadecenoic acid and then to 12,13-dihydroxy-9-octadecenoic acid (12,13-DHOA). From here, there are two bioconversion pathways. The major pathway is: 12,13-DHOA → 12,13,17-trihydroxy-9(S)-octadecenoic acid (THOA) → 12,17;13,17-diepoxy-16-hydroxy-9(Z)-octadecenoic acid (DEOA) → 7-hydroxy-DEOA. The minor pathway is: 12,13-DHOA → 12,13,16-THOA → 12-hydroxy-13,16-epoxy-9(Z)-octadecenoic acid. 12,13,17-THOA has anti-plant pathogenic fungal activity. The tetrahydrofuranyl moiety is known in anti cancer drugs. Strain ALA2 also converts other n-3 and n-6 PUFAs such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) to many new oxygenated unsaturated fatty acid products. All of these new products have high potential for antimicrobial agents or biomedical applications. We also screened 12 *Mortierella* fungal strains from the ARS Culture Collection for the production of bioactive fatty acids such as dihomo-gama-linolenic acid (DGLA) and arachidonic acid. All of the strains tested produced AA and DGLA from glucose or glycerol. The top five AA producers (mg AA/g CDW) were in the following order: *M. alpina* > *M. zychae* > *M. hygrophila* > *M. minutissima* > *M. parvispora*. Both AA and DGLA are important natural precursors of a large family of prostaglandin and thromboxane groups.

Key Words: oxygenated fatty acids, antimicrobials, anti-plant pathogenic fungi, bioactive fatty acids, biocatalysis

INTRODUCTION

The United States produces more than 18 billion pounds of soybean oil (SBO) annually with a yearly carryover of more than 300 million pounds. How to utilize this surplus oil effectively becomes a large economic issue in our agricultural community. SBO is a relatively cheap raw material at 22 to 25 cents per pound and is an attractive candidate for bioindustries. The content of unsaturated fatty acids such as oleic and linoleic acids are 22% and 55% for soybean oil. We are trying to convert SBO to value-added products such as physiologically active oxygenated fatty acids. Microorganisms convert oleic and linoleic acids to many oxygenated fatty acids.[1] Many ω–3 and ω–6 PUFAs are converted to their corresponding oxygenated PUFAs.[2,3] All of these new products have high potential for antimicrobial agents or biomedical applications. Recently, in anticipation of the huge production of co-product glycerol from the national biodiesel program, we screened 12 *Mortierella* fungal strains from the ARS Culture Collection for the production of bioactive fatty acids such as dihomo-gama-linolenic acid (DGLA) and arachidonic acid (AA) from both glucose and glycerol. Results of these studies will be reported here.

Oxygenated Fatty Acids

Hydroxy fatty acids are common in nature. In mammals, oxidation of long-chain fatty acids to α-hydroxy fatty acids has been demonstrated in microsomes of brain and other tissues. α-Hydroxy long-chain fatty acids are constituents of brain lipids. β-Oxidation is the major fate of fatty acid metabolism in mammals. During β-oxidation, fatty acids are oxidized in mitochondria by a sequence of reactions in which the fatty acyl chain is shortened two carbon atoms at a time to produce β-hydroxy fatty acids. Recently, β-hydroxy fatty acids became popular health supplements.

Plant systems are also known to produce hydroxy fatty acids. In these cases, the hydroxy groups are usually located in the middle of the fatty acyl chain. These types of hydroxy fatty acids are important industrial materials. Castor and lesquerella oils are the two major sources.

Microorganisms oxidize fatty acids either at the terminal carbon or inside of the acyl chain. These hydroxy fatty acids are classified into three types, namely: monohydroxy, dihydroxy and trihydoxy fatty acids.

MONOHYDROXY FATTY ACIDS

10-Hydroxystearic acids

Nocardia cholesterolicum hydrated oleic acid at the cis

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9 double bond to produce 10-hydroxystearic acid (10-HAS) with greater than 95% yield.[4]

10-Hydroxy-12(Z)-octadecenoic acid

Flavobacterium DS5 converts linoleic acid to 10-hydroxy-12(Z)-octadecenoic acid with 55% yield.[5] Strain DS5 also converts α-linolenic acid to 10-hydroxy-12,15-octadecadienoic acid and converts γ-linolenic acid to 10-hydroxy-6(*Z*),12(*Z*)-octadecadienoic acid. Strain DS5 converts myristoleic acid to 10-keto myristic and 10-hydroxymyristic acids, and similarly, palmitoleic acid to 10-ketopalmitic and 10-hydroxypalmitic acids. It is interesting to find that all unsaturated fatty acids tested are hydrated at the 9,10 positions with the oxygen functionality at C-10 despite their varying degree of unsaturations. The relative activities are in the following order: oleic > palmitoleic > arachidonic > linoleic > linolenic > gamma-linolenic > myristoleic acids. 10-Hydroxy-12(*Z*)-octadecenoic acid decreased muscular tension in rat cardiac muscle.

DIHYDROXY UNSATURATED FATTY ACIDS

A microbial culture, *Pseudomonas aeruginosa* PR3 was found to convert oleic acid to a new compound, 7,10-dihydroxy-8(E)-octadecenoic acid.[6] The yield of DOD was originally at 60% and is now improved to over 90%. The production of DOD from oleic acid is unique in that it involves both hydroxylation and possibly isomerization, an addition of two hydroxy groups at two positions and a rearrangement of the double bond of the substrate molecule. Strain PR3 also converts ricinoleic acid to 7,10,12-trihydroxy-8(*E*)-octadecenoic acid (TOD) through 10,12-dihydroxy-8(*E*)-octadecenoic acid (DHOD) at 35% yield.[7]

Physiological activity tests of DOD revealed that DOD has activity against *Bacillus subtilis* and a common yeast pathogen, *Candida albicans.* DOD inhibited the growth of many pathogenic bacteria including food borne pathogenic bacteria such as *Escherichia coli* ATCC 8739, *Staphylococcus epidermidis* KCTC 1917, *Salmonella typhimurium KCTC* 2515, *Listeria monocytogenes* ATCC19111, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6501. It also inhibited the growth of the following plant pathogenc bacteria: *Pseudomonas syringae pv. sesami* KACC 10649, *Pseudomonas syringae pv. actinidae* KACC 10659, *Erwinia sp.* KACC 10207, *Pseudomonas syringae pv. syringae* KACC 10361, *Ralstonia solanacearum* KACC 10475, *Corynebacterium glutamicum* KACC 10784, *Pseudomonas corrugata* KACC 10141, *Xanthomonas axonopodis pv. citri* KACC 10443, *Xanthomonas campestris* KACC 10490, and *Clavibacter michiganesis subsp. michiganensis* KACC 20122.

Strain PR3 also converted linoleic acid to many isomeric 9,10,13 (9,12,13)-trihydroxy-11E (10E)-octadecenoic acids.[8] TOD showed anti-plant pathogenic fungal activities, especially on the rice blast fungus. DOD was tested for its application in the production of polyurethane rigid foam, and in skin cares products. Palmitoleic acid was converted to 7,10-dihydroxy-8(*E*)-hexadecenoic acid.

TRIHYDROXY UNSATURATED FATTY ACIDS

We also discovered the production of a new compound, 12,13,17-trihydroxy-9(*Z*)-octadecenoic acid (12,13,17-THOA) from linoleic acid by a new microbial culture which was isolated from a dry soil sample collected from McCalla, Alabama.[9] The strain was identified as *Clavibacter* sp. ALA2 and later re-classified as *Bacillus megaterium* ALA2. The yield of 12,13,17-THOD from linoleic acid was 35%.

Production of trihydroxy unsaturated fatty acids in nature are rare. These compounds are all produced in trace amounts by plants. Mixed hydroxy fatty acids were isolated from the *Sasanishiki* variety of the rice plant, which suffered from the rice blast disease and were shown to be active against the fungus. Trihydroxy fatty acids were also isolated from potato, *Colocasia antiquorum* inoculated with black rot fungus, *Ceratocystis fimbriata*, and showed anti-black rot fungal activity.[10]

Other than extraction from plant materials, our discovery is the first report on production of trihydroxy unsaturated fatty acids by microbial transformation. The biological activity of 12,13,17-THOA was found[11] to inhibit the growth of the following plant pathogenic fungi: *Erisyphe graminis f. sp. tritici* (wheat powdery mildew); *Puccinia recondita* (wheat leaf rust); *Phytophthora infestans* (potato late blight); and *Botrytis cinerea* (cucumber botrytis). It seems that the position of the hydroxy groups on the fatty acids molecule plays an important role in the activity against certain specific plant pathogenic fungi.

OTHER OXYGENATED FATTY ACIDS FROM LINOLEIC ACID

In addition to the main product 12,13,17-THOA, the strain ALA2 system also produced the following products from linoleic acid[12]: two tetrahydrofuranyl fatty acids (THFAs), 12-hydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid and 7,12-dihydroxy-13,16-epoxy-9(*Z*)-octadecenoic acid; two diepoxy bicyclic fatty acids, 12,17;13,17-diepoxy-9(*Z*)-octadecenoic acid (DEOA) and 12,17;13, 17-diepoxy-7-hydroxy-9(Z)-octadecenoic acid (hDEOA). Tetrahydrofuranyl compounds are known to have anticancer activity. The diepoxy bicyclic fatty acids are completely new chemical entities. With many functionality groups on the molecules, their application in biomedical as well as specialty chemical industries is expected. A small amount of 12,13,16-trihydroxy-9(*Z*)-octadecenoic acid (12,13,16-THOA) and 12,13-dihydroxy-9(*Z*)-octadecinoic acid (12,13-DHOA) were also detected in the bioconversion system.

The bioconversion pathway from linoleic acid to many oxygenated fatty acids were established.[13,14] The main pathway is: Linoleic acid → 12,13-epoxy-9-octadecenoic acid → 12,13-DHOA → 12,13,17-THOA → DEOA → hDEOA. And the minor pathway is branched out from 12,13-DHOA →12,13,16-THOA →12 hydroxy-THOA → 7,12-dihydroxy-THOA.

Conversion of other ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) by strain ALA2

Strain ALA2 also converted other ω–3 and ω–6 polyunsaturated fatty acids (PUFAs) into a variety of oxylipins. For examples, it converted α-linolenic acid to novel

THFAs: 13,16-dihydroxy-12, 15-epoxy-9(Z)-octa decenoic acid (13,16-dihydroxy-THFA) and 7,13,16-trihydroxy-12, 15-epoxy-9(Z)-octadecenoic acid (7,13, 16-trihydroxy-THFA).[2] 7,13,16-Trihydroxy-THFA is a new chemical entity with trihydroxy fatty acid containing a cyclic structure.

Strain ALA2 also bioconverts other ω–3 PUFAs such as EPA and DHA and ω–6 PUFAs such as γ-linolenic acid and arachidonic acid into new products.[3] From EPA, 15, 18-dihydroxy-14,17-epoxy-5(Z),8(Z),11(Z)-eicosatrienoic acid and from DHA, 17, 19-dihydroxy-16,18-epoxy-4(Z),7(Z),10(Z),13(Z)-docosatetoraenoic acid were obtained. Strain ALA2 converts γ-linolenic acid into three products, two major products: 12,17; 13,17-diepoxy-6,9-octadecadienoic acid, and 12,13,17-trihydroxy-6,9-octadienoic acid; and one minor product, 12-hydroxy-13,16-epoxy-6,9-octadecadienoic acid. Arachidonic acid was also converted into three products: 14,19;15,19-diepoxy-5(Z),8(Z),11(Z)-eicosatrienoic acid; 14-hidroxy-15,18-epoxy-5(Z),8(Z),11(Z)-eicosatrienoic acid; and 14,15,19-trihydroxy-5(Z),8(Z),11(Z)-eicosatrienoic acid. It is interesting to note that the structures of the products are remarkably different between those obtained from n-3 and from n–6 PUFAs. It seems that substrate PUFAs with a double bond at the ω–3 position affects the outcome of the bioconversion products by ALA2 enzymes. Strain ALA2 enzymes may be able to recognize the ω–3 carbon terminal of PUFAs and proceed with hydroxylation and cyclization accordingly. It is known that hydroxylation at the ω–1, ω–2, and ω–3 positions are catalyzed by cytochrome P-450.

Production of arachidonic acid by Mortierella

Arachidonic acid is an n–6 polyunsaturated fatty acid. Recently, AA and DHA are supplemented in infant formula with believe that these two fatty acids are important for infant growth and development. AA is naturally found in egg yolk and meat, whereas DHA is from fish oil. However, both AA and DHA in commercial use are mainly produced by the fermentation of fungi and microalgae. In this communication, we compare the production of AA in different Mortierella strains.

Twelve *Mortierella* strains: *M. alpina* NRRL 6302, *M. claussenii* NRRL 2760, *M. elongata* NRRL 5246, *M. epigama* NRRL 5512, *M. humilis* NRRL 6369, *M. hygrophila* NRRL 2591, *M. minutissima* NRRL 6462, *M. multidivaricata* NRRL 6456, *M. nantahalensis* NRRL 5216, *M. parvispora* NRRL 2941, *M. sepedonioides* NRRL 6425, and *M. zychae* NRRL 2592 were screened for their production of arachidonic acid (AA) and dihomo-γ-linolenic acid (DGLA). All of the strains tested produced AA and DGLA from glucose. The total fatty acid 125 mg/g cell dry weight (CDW) and fatty acids composition for AA (19.63%) and DGLA (5.95%) in the mycelia of *M.* alpina grown on glucose were compatible with those reported in the literature. The top five AA producers (mg AA/g CDW) were in the following order: *M. alpina* > *M. zychae* > *M. hygrophila* > *M. minutissima* > *M. parvispora*. The top five CDW productions were: *M. multidivaricata* > *M. parvispora* > *M. humilis* > *M. zychae*. And the top five strains for total fatty acids production (mg /g CDW) were: *M. alpina* > *M. parvispora* > *M. hygrophila* > *M. zychae* > *M. minutissima* > *M. epigama*. In anticipation of a huge production of the co-product glycerol from the national biodiesel program, these 12 strains were screened for their production of AA and DHLA from glycerol. The top five AA producers from glycerol (mg AA/CDW) were in the following order: *M. parvispora* (18.2) > *M. claussenii* (15.9) > *M. alpina* (15.0) > *M. zychae* (14.2) > *M. minutissima* (13.0). The top five dry mycelia weights productions were: *M. zychae* (0.346) > *M. epigama* (0.325) > *M. hygrophila* (0.279) > *M. humilis* (0.268) > *M. minutissima* (0.253). And the top five strains for total fatty acids production (mg /g CDW) were: *M. claussenii* (101) > *M. parvispora* (101) > *M. minutissima* (94.9) > *M. hygrophila* (86.0) > *M. maltidivaricata* (83.0).

CONCLUSIONS

In conclusion, polyunsaturated fatty acids can be bioconverted to several unique oxygenated fatty acid products with various biological functions. These metabolites include prostaglandins, prostacyclines, thromboxanes, leukotrienes, lipoxins, and hydroxy, hydroperoxy, and epoxy fatty acids. The yeast *Dipodascopsis uninucleata* UOFS-Y128 can transfer exogeneous arachidonic acid to a stable 3-hydroxy-5,8,11,14-eicosatetrae noic acid (3-HETE), a compound possessing signal trasduction activity in human neutrophils. Recently, it was reported that low doses of 9,(12)-oxy-10,13-dihydroxy-stearic acid and 10,(13)-oxy-9,12-dihydroxy-stearic acid isolated from corn stimulated breast cancer cell proliferation *in vitro* and disrupted the estrous cycle in female rats. These dihydroxy-THFA inhibited breast cancer and prostate cancer cell proliferation at high doses. However, little is known about biological functions and chemical properties of hydroxy-unsaturated THFA. Therefore, it will be interesting to find out the physiological functions and industrial application of these novel hydroxy-unsaturated THFAs. Research and development of products in these areas have a great future.

AUTHOR DISCLOSURES

Ching T Hou, no conflicts of interest.

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