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MICROBIAL TRANSFORMATION OF LIPIDS: SELECTIVE HYDRATION OF OLEIC ACID

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تميزت ميكروبات نوكارديا سبيسس ونوكارديا اورانتيا وميكوبكتريم فورتيتم بقدرتها على هيدرة حمض الاوليك. وثبت من الدراسة أن الميكروبات المذكورة تخصصت فى هيدرة الرابطة الثنائية-سيس الخاصة بحمض الاوليك وحتى فى وجود احماض دهنية اخرى مشبعة وغير مشبعة. وقد ثبت ان استرات احماض الاستياريك وبالمتيك ولينوليك لا تتأثر بانزيم الهدراتيز الموجود فى الثلاث ميكروبات رغم تأثيرها الواضح على حمض الاوليك بنسبة ٨٨٪ وتحويله الى ١٠-هيدروكسى ستياريك والذى ثبت باستخدام كروماتوجرافيا الغاز. هذا الانزيم يمكن ان يميز بين الاحماض المشبعة والاحماض ثنائية الرابطة والاحماض احادية الرابطة (ترانس) مثل حمض الياديك.

هذه الانزيمات أمكنها التمييز بين الاحماض التى لها نفس الطول سواء المشبعة (١٨:٠) مثل حمض ستياريك والغير مشبعة (١٨:١ ترانس) مثل حمض الياديك و (١٨:٢) مثل حمض لينوليك و(٢٢:١) مثل اريوسيك وكذلك الاحماض الاقصر منها المشبعة (١٦:٠) مثل حمض بالمتيك والغير مشبعة (١٦:١) مثل حمض بالميتوليك وايضا (١٤:١) مثل حمض ميريستوليك.

وتظهر فائدة البحث فى امكانية اكسدة وفصل حمض الاوليك فى وجود خليط من الاحماض الدهنية والزيوت النباتية التى تحتوى على احماض دهنية غير مشبعة.

The cultures Nocardia sp. NRRL 5646, Nocardia aurantia ATCC 12674, and Mycobacterium fortium AM 53378 were identified as being capable of metabolizing oleic acid. These organisms selectively hydrated the cis-double bond of oleic acid in the presence of other fatty acdis like oleic:linoleic (1:1, w/w), oleic:stearic (9:1, w/w), oleic:linoleic:stearic (2:2:1, w/w), and oleic:linoleic: palmitic (2:2:1, w/w). Based on gas chromatography of the fatty acid methyl esters, it was found that stearic, palmitic, and linoleic acids were unreactive with the hydratase enzyme of the three microbes and in all cases 80-88% of oleic acid was consumed. Thus, the hydratase enzyme was able to selectively and quantitatively hydrate oleic acid and discriminate among monoenoic, dienoic and saturated fatty acids This work provides a mean of oxidatively removing oleic acid from among a mixture of other mono and polyunsaturated vegetable oil fatty acids. We believe that the selected organisms have the abilities to transform oleic acid and to selectively utilize that compound from similar mixtures as well.

INTRODUCTION

Fatty acids of plants, animal and microbial origin generally contain even number of carbon atoms in straight chains with carboxyl group at one extremity in addition to one or more cis-double bond at specific position[1]. Plant and microbial fatty acids may contain other functional groups such as hydroxy and keto groups[1], and trans-double bonds as in trans-3-hexadecenoic acid found in plant chloroplast lipids[2,3]. Some straight chain even number fatty acids with 10-30 carbon atoms posses cis-double bond[2,3]. Animal and plant lipids frequently contain families of mono-enoic fatty acids with similar terminal structures, but with different length hydrocarbon chains[2]. Commercial hydrogenation of fats and oils resulted in production of unsaturated fatty acids[4]. Double bond oxidation may result in formation of mono-

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and/or dihydroxy, keto and apoxy derivatives[5]. 10-Hydroxy and 10-ketostearic acids were prepared by hydrating the double bond of octadecenoic acids[5,6]. Trans-double bond substrates were shown to resist hydration by *Pseudomonas* hydratase[6]. *Mucor* species has a hydrating activity to transform trans-2-alkenoic acid into L-3-hydroxyalkanoic acid in a reversible fashion[7]. *Pseudomonas* enzyme preparation catalyzed the interconversion of oleic and 10-D-hydroxystearic acid[8], but did not catalyze formation of olefinic acid from 9-D-hydroxystearic acid[8].

This report deals with selective metabolism of oleic acid and oleic acid/fatty acids mixtures in different proportions by utilizing the hydrating activity of *Nocardia aurantia* ATCC 12674, *Nocardia sp* NRRL 5646, and *Mycobacterium fortitum* AM 53378.

EXPERIMENTAL

Microorganisms

Nocardia aurantia ATCC 12674, *Nocardia sp* NRRL 5646, and *Mycobacterium fortitum* AM 53378 were obtained from the culture collection at the college of pharmacy, University of Iowa which were originally purchased from North Regional Research Laboratories (NRRL) and American Type Culture Collection (ATCC), and were maintained at 4°C on nutrient agar slants.

Fatty acids

Oleic acid was obtained from Fisher Scientific; stearic, palmitic, and linoleic, myristoleic, palmitoleic, eliadic, erucic, nervonic acids and borontriflouride-methanol complex (12%) were purchased from Sigma Chemical Co., all fatty acids found 99% pure by GC. The fatty acids and mixtures used in the experiments consisted of 100% oleic, oleic:linoleic (1:1, w/w), oleic:stearic (9:1, w/w), oleic: linoleic:stearic (2:2:1, w/w), and oleic: linoleic:palmitic (2:2:1, w/w).

Instruments and general procedure

Gas chromatography (GC) was performed on a Hewlett-Packard 5890A gas chromatograph linked to a Hewlett-Packard 3390 integrator. Nitrogen was used as a carrier and a make-up gas, hydrogen and air were used as a source of energy for the flame ionization detector (FID). A Supelcowax capillary column 30m x 0.32 ID was used (Supelco Inc, Bellafonte, PA, USA) with 0.2 μm film thickness. Nitrogen (preheated and purified), hydrogen, air, and nitrogen make-up gas were used at flow rates of 30, 20, 260, and 10 ml/minute, respectively. Column, injector, and detector temperatures were maintained at 180-210°C (5°/min.), 220°C, 300°C, respectively. column head pressure was 35 psi for carrier gas, and hydrogen pressure was 30 psi.

Fermentation procedure

The microbial cultures were grown using the standard two-stage fermentation protocol[9,10] in a medium consisted of dextrose 2%, yeast extract 0.5%, soy bean 0.5%, NaCl 0.5%, and K_2HPO_4 0.5% and was adjusted to pH 7 before sterilization for 15 minutes. Stage-II culture was started by transferring 3 ml of the 48 hr-old stage-I culture into 25 ml fresh medium. The cultures were incubated for 24 hr. at 28°C, 250 rpm before addition of a 100 mg of the fatty acid or fatty acids mixture (in 100 μl DMSO). Both culture and substrate control were performed under the same culture conditions and the experiments were done in duplicates.

Sampling and analysis

The fermentation mixtures were sampled after 6, 12, 24, 72, 96, 120 and 168 hours. The one-ml samples were extracted with one ml of EtOAc-CH_3OH (9:1, v/v) by gentile shaking (three times), the organic layers were transferred to a 5 ml vial and evaporated to dryness under nitrogen. The dried materials were methylated[11,12] by addition of 200 μl of borontriflouride-methanol complex 12% and heated over steam bath for three minutes (completion of the reactions was monitored by spotting the reaction solution on TLC plates). The reaction mixtures were analyzed by gas chromatography after evaporation to dryness under nitrogen gas and reconstituted in one ml

of methanol.

RESULTS AND DISCUSSION

Nocardia aurantia ATCC 12674, *Nocardia sp* NRRL 5646, and *Mycobacterium fortitum* AM 53378 were shown to metabolize cis-9-octadecenoic (oleic) to 10-oxo-octadecanoic acid in high yield[13]. Cis-9-double bond of oleic acid was hydrated to give a saturated fatty acid which was further oxidized to a keto function at C_{10} position[13] of the fatty acid present in the mixtures oleic:linoleic (1:1, w/w), oleic:stearic (9:1, w/w), oleic:linoleic:stearic (2:2:1, w/w), and oleic:linoleic:palmitic (2:2:1, w/w). This demonstrated the selective hydration of oleic acid double bond in the presence of other fatty acids. Quantitative study using GC analysis of palmitic, stearic, oleic and linoleic acids standards were shown at R_t 2.86, 4.63, 4.84, and 5.12 minutes (Figure 1). The reaction mixtures displayed the ability of the microorganisms to metabolize 80-88% of oleic acid within 7 days based on peak areas and standard curves of the corresponding fatty acids. The concentration of each of palmitic, stearic, and linoleic acid did not change significantly.

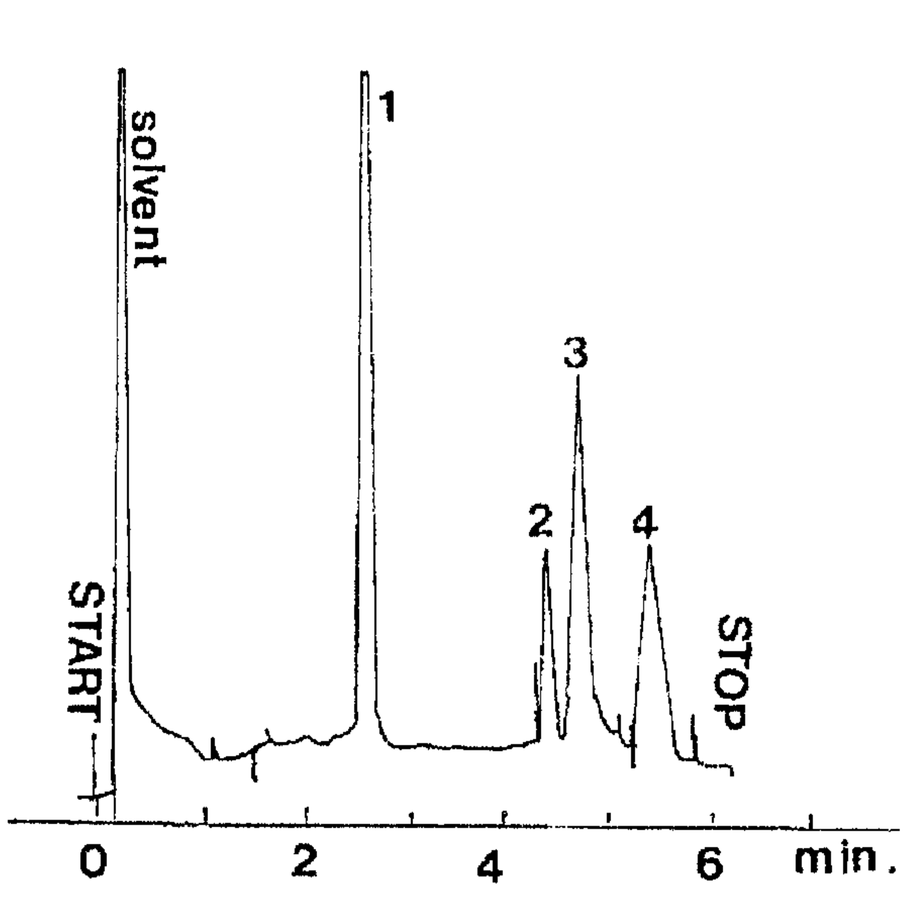

Fig. 1: Gas Chromatographic (GC) profile of the fatty acid methyl esters. Palmitic acid **1**, Stearic acid **2**, Oleic acid **3**, and Linoleic acid **4**.

However, the percentage yield of the product, 10-oxo-stearic, after 168-hours culture incubation with *Nocardia sp.*, *N. aurantia* and *M. fortitum* was 25% (P/O/L), 50% (O/L), and 50% (O/L), respectively.

The three microbes were able to transform oleic acid (18:1 cis) to 10-oxo-stearic acid in the presence of other fatty acids. However, these microorganisms did not perform any activity against eliadic acid (18:1 trans), and the dienoic acid (18:2). Fatty acid of the same length like stearic (18:0) or shorter length with or without double bond like myristoleic acid (14:1), palmitoleic acid (16:1), and palmitic acid (16:0) were not attacked by the hydratase enzymes of the microorganisms. Longer chain fatty acids such as erucic acid (22:1), and nervonic acid (16:0) were not affected. Stearic and palmitic acids can not be hydrated because of the lack of any double bond in their structures. Thus, saturated fatty acids were shown to be stable against the activity of these enzymes. However, it was observed that the transformation of oleic acid into 10-oxo-stearic was increased in the presence of linoleic acid which may be attributed to linoleic acid double bonds which resist the hydration by being inaccessible to the enzyme surface. The lipase present in the three microorganisms have a unique specificity in hydrating cis-9-unsaturation (18:1) only not cis-cis-9, 12-unsaturated fatty acid (linoleic acid). The enzymes were able to discriminate between saturated (18:0) and (16:0), monoenoic (14:1), (16:1), (18:1 trans), and (18:1 cis) and dienoic (18:2) fatty acids. Isolation and characterization of these enzymes is underway.

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