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ORIGINAL PAPER

Selective Microbial Degradation of Saturated Methyl Branched-Chain Fatty Acid Isomers

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Abstract Three strains of *Pseudomonas* (*P.*) bacteria were screened for their capabilities of degrading chemically synthesized saturated branched-chain fatty acids (sbc–FA). Mixtures of sbc–FA with the methyl-branch located at various locales along the fatty acid were used as a carbon feedstock in shake-flask culture. Utilization (and hence degradability) of the sbc–FA was monitored based on positive bacterial growth, fatty acid recovery rates and chromatographic (gas chromatography (GC) and GC-mass spectroscopy (MS)) analysis of the recovered carbon source. *P. putida* KT2442 and *P. oleovorans* NRRL B-14683 were both able to grow on sbc–FA utilizing 35 wt% and 27 wt% of the carbon source, respectively after 144 h. In contrast, *P. resinovorans* NRRL B-2649 exhibited the most efficient use of the carbon source by utilizing 89 % of the starting material after 96 h resulting in a cell dry weight (CDW) of 3.1 g/L. GC and GC–MS analysis of the recovered carbon source revealed that the bacterial strains selectively utilized the isostearic acid in the sbc–FA mixture, and a new group of C_{10}, C_{12}, C_{14} and C_{16}-linear and/or branched-chain fatty acids (approximately 4–29 wt%) were formed during degradation.

Keywords Biodegradability · Branched-chain fatty acid isomers · Isostearic acid · Lubricants

Introduction

Environmental concerns have dictated a shift in focus to the development of biocompatible substitutes for many non-renewable materials. Historically, materials such as fuels, polymers, lubricants and many other chemicals have been produced from non-renewable petroleum-based feedstocks. The dilemma has been that petroleum-based materials, while generally cheaper to produce than bio-based products, tend to resist the typical degradation processes found in nature (e.g., photolysis: the chemical process by which molecules are broken down into smaller units through the absorption of light, and hydrolysis: the chemical process in which molecules are broken-down by the addition of water) resulting in problems in waste management [1–3]. In addition, the little breakdown that does occur often results in greenhouse gas emissions that have been determined to contribute to ozone layer depletion, as well as have other deleterious effects [4]. For this reason, efforts are underway to develop sustainable replacements for many of the petroleum-based materials currently in use and to do it cost-effectively. Success in this area will provide materials that can be produced from readily available plant and animal materials, and that can be naturally degraded in a carbon-neutral process.

The most effective means of breaking down any material in nature is through biodegradation. The term "biodegradable" implies that a material is subject to microbial

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attack and mineralization to CO_2, and water through the action of specific enzymes present in a bioactive environment. Standards such as ASTM D 6400 [5], EN 13432 [6], ISO 17088 [7] among others have been established as benchmarks for determination of biodegradability and although, each of these standards is unique, their fundamental requirements for determining complete biodegradation are the microbial assimilation of the test material to CO_2, water and biomass whereas, 60–90 % of the carbon is converted to CO_2 in less than 180 days (depending on the specific standard). In addition, the degradation rates should approximate those of natural materials such as leaves, paper, grass and food scraps, and the resultant compost should have no negative impacts on plants. Many potential bio-based materials derived from natural sources including fats and oils for the lubricant industry [8], and certain biopolymers such as polylactic acid (PLA; [9]), lignocellulosics [10] and polyhydroxyalkanoates (PHA; [11, 12]) among others are being chemically modified to suit specific applications. While it is generally assumed that materials, whose origins are from natural sources are "environmentally benign," once chemical modifications occur there is no guarantee that the newly synthesized materials will exhibit the same biodegradable character as the parent molecule, even in microbially active realms. Therefore, it is imperative that these materials be tested to ascertain their potential environmental impact upon disposal.

Typically, linear fatty acids are catabolized through the β-oxidation pathway 2 carbon units at a time; however, the inclusion of methyl branches at different points along the alkyl chain inhibits the typical β-oxidation pathway and necessitates the presence of new or modified catabolic pathways in order to degrade these materials. One strategy that has been noted for the breakdown of methyl-branched fatty acids is α-oxidation which is a process by which fatty acids are shortened at the carboxyl-end by a single carbon atom [13]. This pathway, while efficient is generally associated with the peroxisomes of mammalian cells [14] and not in the typical microbes that are naturally prevalent in the environment. However, studies have been reported that show the ability of certain bacterial strains to break down methyl-branched saturated alkanoic acids. As early as 1959 it was known that *Pseudomonas (P.) aeruginosa* was capable of metabolizing certain branched alkanes by simply monitoring bacterial growth [15]. Later, other strains of bacteria were reported to have the capability of breaking down methyl-branched alkanoic acids [16]. By using 3-methylvaleric acid (3-MVA) as a growth substrate, 3 bacterial strains were discovered that possessed the metabolic capability to catabolize 3-MVA. The first, *P. citronellolis* utilized a β-methyl activation sequence involving CO_2 fixation, analogous to that seen in the isovalerate pathway to break down 3-MVA [16] ,while the second strain, a *Mycobacterium* isolate used α-oxidation to convert 3-MVA to 2-methylbutyrate, which was then assimilated through the isoleucine pathway [16]. Lastly, an *Arthrobacter* isolate metabolized 3-MVA via ω-oxidation to produce 3-methylglutarate that was degraded by the 3-hydroxy-3-methylglutarate pathway [16]. In each case, unique bacterial strains utilized the methyl-branched substrate demonstrating different metabolic variations that could be used in degrading these molecules.

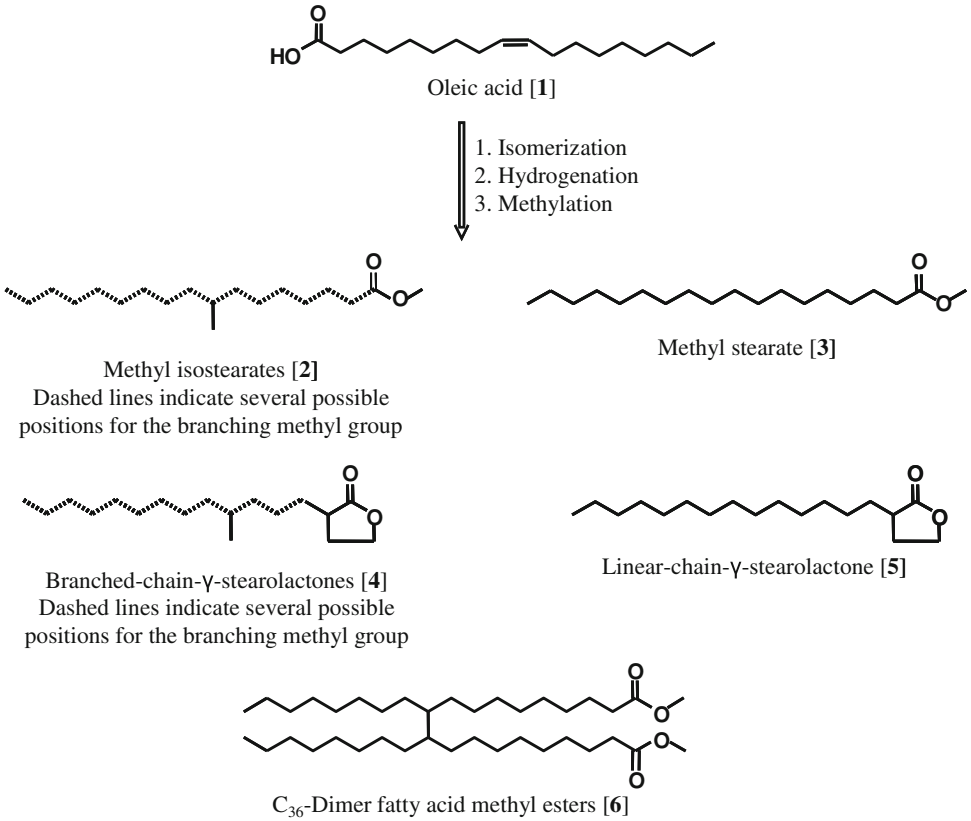
Isostearic acid (i.e., a mixture of saturated methyl branched-chain fatty acid isomers) is a bio-based product formed through a chemical process known as skeletal isomerization of monounsaturated fatty acids [17–21]. Isostearic acid has a hydrophilic carboxylic acid head group and a hydrophobic tail group with a methyl group located at various positions along the alkyl chain (Scheme 1, [**2**]). In contrast to stearic acid (Scheme 1, [**3**]), a saturated linear chain fatty acid that is a solid at room temperature, isostearic acid is a liquid at room temperature. It has been found to possess good low-temperature properties, excellent lubricity, oxidative stabilities and a high-viscosity index number [22]. Isostearic acid thus has a potential for application in the lubricant, cosmetic, emollient, and hydraulic fluid arenas. In this report, we describe our efforts to demonstrate the biodegradable nature of isostearic acid by utilizing 3 separate strains of *Pseudomonas* that are known to have the capacity of using both saturated and unsaturated linear and/or branched-chain fatty acids for their metabolic processes specifically, as they relate to cell growth and polyhydroxyalkanoate biosynthesis [23–25]. By monitoring bacterial growth, carbon source utilization and content, we reveal selective utilization of isostearic acid which is indicative of biodegradability under the conditions employed. Conversely, we show that the branched chain γ-stearolactones, linear chain γ-stearolactones and C_{36}-dimer fatty acids also present in the carbon source were resistant to bacterial breakdown under the conditions employed.

Materials and Methods

Materials

All of the chemicals used in this work were obtained from commercial sources and used without further purification. Oleic acid (91.2 wt% $C_{18:1}$, 6.1 wt% $C_{18:2}$, 2.7 wt% saturated fatty acids) and methanol were purchased from the Aldrich Chemical Company (Milwaukee WI). All salts used as fermentation media components were purchased from the Sigma Chemical Company (St. Louis, MO). Sulfuric acid was obtained from the Mallinckrodt Baker Co. (Phillipsburg, NJ). Pricat 9910 nickel (1 % *w/w*) on

Scheme 1



silica solid catalyst (Ni-SiO_2) was a gift from Johnson Matthey Incorporated (West Chester, PA). Ferrierite zeolite (HSZ-720KOA, potassium (K^+), 17.5 mol/mol SiO_4/AlO_4) was from the Tosoh Co. (Tokyo, Japan). Methyl tridecanoate ($C_{13:0}$) and GLC 420-D11-A ($C_{8:0}$-$C_{18:3}$ FAME) reference standards were from Nu-Chek Prep, Inc (Elysian, MN). *P. resinovorans* NRRL B-2649 and *P. oleovorans* NRRL B-14683 were both obtained from the National Center for Agricultural Utilization Research, Agriculture Research Service, United States Department of Agriculture (Peoria, IL). *P. putida* KT2442 was kindly supplied by Professor Richard Gross, Herman Mark Professor in the Department of Chemical and Biological Science, Polytechnic Institute of New York University (Brooklyn, NY).

Synthesis of Crude Saturated Branched-Chain Fatty Acid Isomers (sbc–FA)

The detailed procedure used to convert the unsaturated linear chain fatty acid (oleic acid) to the crude saturated branched-chain fatty acid isomeric mixture (sbc–FA) was reported previously [17, 18]. The crude sbc–FA were synthesized in a two step process, starting from commercially available oleic acid at quantitative yield for each step (Scheme 1). First, the oleic acid was isomerized under high-pressure and high-temperature in the presence of modified H-ferrierite zeolite, and a small amount of water to give the unsaturated branched-chain fatty acid mixture (ubc-FA). The ubc-FA intermediate was then pressurized with 400 psi hydrogen gas in the presence of 1 % (*w/w*) Ni-SiO_2 at 170 °C for 3 h. The reactor was then depressurized and the Ni-SiO_2 was separated from the sbc–FA by filtration. In the previous papers, Palladium on carbon catalyst (Pd/C) was used to hydrogenate ubc–FA [17, 18]; however, in this instance, we decided to use a nickel catalyst, because of the large quantity of material needed and using Pd/C would not have been cost-effective. The hydrogenated and methylated sbc–FA products were characterized by GC–MS and, the relative concentration of the identified FAME was determined by GC with a flame ionization detector (FID) as previously reported [17, 18]. The relative concentration was determined using an internal standard ($C_{13:0}$) and was based on the FID linear response for fatty acid methyl esters (FAME) [26, 27].

Shake-Flask Parameters

Three separate bacterial strains *P. resinovorans*, *P. oleovorans*, and *P. putida* were utilized in this work. Cryogenically preserved stock cultures of each organism were prepared as described previously [28]. All degradation

experiments were conducted on the shake-flask scale in 500 mL of Media E* [29] (starting pH 7.0) in 1-L unbaffled Erlenmeyer flasks. Media E* salts and carbon source (sbc–FA synthesis described above) were prepared resulting in growth media containing 1 % (*w/v*) sbc–FA. The media was then autoclaved to sterilize. Each of the aforementioned bacterial strains was tested for its ability to degrade sbc–FA as a function of time. The inocula for each of the shake-flask experiments were prepared by inoculating 50 mL of Luria–Bertani (LB) broth (1 % tryptone, 0.5 % NaCl, 0.5 % yeast extract), with 1.5 mL of bacteria from frozen stock cultures and incubating the cultures at 30 °C, 250 rpm for 24 h. After 24 h, the LB cultures were removed from the shake incubator and 0.5 mL of LB bacterial culture was transferred into each 500-mL test flask, and each flask placed back into the shake incubator at the previously noted conditions. At the appropriate times, flasks were harvested as described below at either 24 h or 48 h intervals.

Shake-Flask Harvest Conditions

Single flasks were harvested in a manner that would allow re-isolation of residual sbc–FA and/or any fatty acid derivatives, and allow the gravimetric determination of bacterial cell dry weight (CDW) over time. To harvest, the entire culture was placed into a large separatory funnel and extracted twice with 300 mL of hexane. After each extraction, the hexane/organic fraction (containing the residual carbon source) and the aqueous fraction (containing the bacterial cells) were separated. The organic layers from the two extractions were combined ($\sim$600 mL total) and excess sodium sulfate (Na_2SO_4) was added to remove any residual water, after which the Na_2SO_4 was removed by filtration through Whatman #2 filter paper. The organic fractions were then placed into separate tared round-bottom flasks and the hexane was removed by rotary evaporation leaving the residual carbon source behind. The round-bottom flasks were placed under vacuum overnight to remove any residual hexane and were then reweighed. The amount of residual carbon source was determined gravimetrically by difference. The residual carbon sources were analyzed by GC and GC–MS as described above to determine preferential carbon source utilization, within the sbc–FA isomeric mixtures. The aqueous layers were centrifuged (8,000$\times g$, 4 °C for 15 min) to pellet the bacterial cells. The cells were washed once in deionized water and re-centrifuged under the same conditions, after which the cells were frozen and lyophilized to dryness ($\sim$24 h). Once dry, the CDW for each of the cultures were determined gravimetrically.

Results and Discussion

The objective of this work was to determine, if fatty acids maintained biodegradability after chemical modification to sbc–FA (methyl-branching), in order to gain a better understanding of the potential impact of sbc–FA upon disposal. One of the easiest methods to determine, whether a specific bacterial strain can utilize and hence degrade a particular material is by monitoring its growth, when the material in question is used as the sole carbon source. In this instance the crude sbc–FA mixture was used as a feedstock

Table 1 Carbon source content of the recovered saturated branched chain-FA after exposure to bacterial growth[a]

Entry	Bacterial strain	Time [h]	Percentage composition from GC					
			Methyl isostearates [**2**[b]]	Methyl stearate [**3**[b]]	γ-Stearolactones [**4**[b] & **5**[b]]	C_{36}-dimer FAME [**6**[b]]	Unknowns	C_{10},C_{12},C_{14},C_{16}-branched-FAME
1	None	0	75.4	2.1	8.6	13.9	0	0
2	*P. resinovorans*	48	62.4	1.7	11.0	22.0	2.9	0
3	*P. resinovorans*	96	48.0	1.7	17.0	22.4	10.9	0
4	*P. putida*	48	51.3	1.9	10.1	18.2	0	18.5
5	*P. putida*	96	40.4	1.8	10.8	17.6	0	29.4
6	*P. putida*	144	40.4	1.5	11.0	18.1	0	29.0
7	*P. oleovorans*	48	71.5	2.3	8.8	13.7	0	3.8
8	*P. oleovorans*	96	65.9	2.2	9.1	14.2	0	8.6
9	*P. oleovorans*	144	46.0	1.9	10.0	16.3	0	25.8

FAME fatty acid methyl esters, *FA* fatty acids, *P Pseudomonas*

[a] Starting fatty acids are sbc–FA. Products were methylated and then analyzed by GC. Results were compared against internal standard methyl tridecanoate (C13:0)

[b] Referring to Scheme 1

for 3 specific strains of *Pseudomonas*, whose growth and sbc–FA consumption was monitored in order to assess the relative rates of carbon source utilization. Table 1, entry 1 lists the product distribution obtained by GC analysis for the crude sbc–FA before exposure to bacterial growth. The parental material included both monomeric (75.4 wt%) isostearic acid **[2],** Scheme 1; 2.1 wt% stearic acid **[3],** Scheme 1; and 8.6 wt% γ-stearolactones **[4]**, **[5],** Scheme 1 as well as oligomeric (13.9 wt% dimer fatty acids **[6],** Scheme 1) products. GC–MS analysis showed that the monomeric components had the following molecular ions ($M^{+\bullet}$) at *m/z* (**[2]** $M^{+\bullet} = 298$; **[3]** $M^{+\bullet} = 298$; **[4]** $M^{+\bullet} = 282$; **[5]** $M^{+\bullet} = 282$). In our previous work [17, 18, 22], we reported that the branched-chain mixtures contained small amounts of hydroxy-methyloctadecanoates. However, after further analysis of the mixture, we found that the product was in fact γ-branched-chain stearolactones (Scheme 1, **[4]**), but not hydroxy-methyloctadecanoates. Matrix-assisted laser desorption/ionization-time of flight (MALDI-ToF) showed that the dimer (Scheme 1, **[6]**) had a molecular weight of 595. We used this carbon source mixture in the present study, because as mentioned previously, this mixture has excellent lubricity and selected properties [22]. Fig. 1a, b show the relative rates of carbon source utilization and the CDW among the 3 bacterial strains tested. *P. putida* and *P. oleovorans* utilized 35 % and 27 % of the starting sbc–FA carbon source after 144 h resulting in CDW of 0.76 g/L and 0.74 g/L, respectively. These results demonstrated that these 2 bacterial strains both had the ability to utilize at least a portion of the carbon substrate, but the utilization was accompanied by a relatively long lag phase resulting in prolonged incubation times. In contrast, *P. resinovorans* utilized 89 % of the sbc–FA substrate in 96 h resulting in a CDW of 3.1 g/L. These data showed that *P. resinovorans* was better equipped genetically to handle growth on the crude sbc–FA mixture resulting in more efficient carbon source utilization and higher CDW. This was not entirely surprising in that one of our previous reports showed the ability of *P. resinovorans* to convert isostearic acid to methyl-branched medium-chain-length (*mcl*-) polyhydroxyalkanoate biopolymers [23].

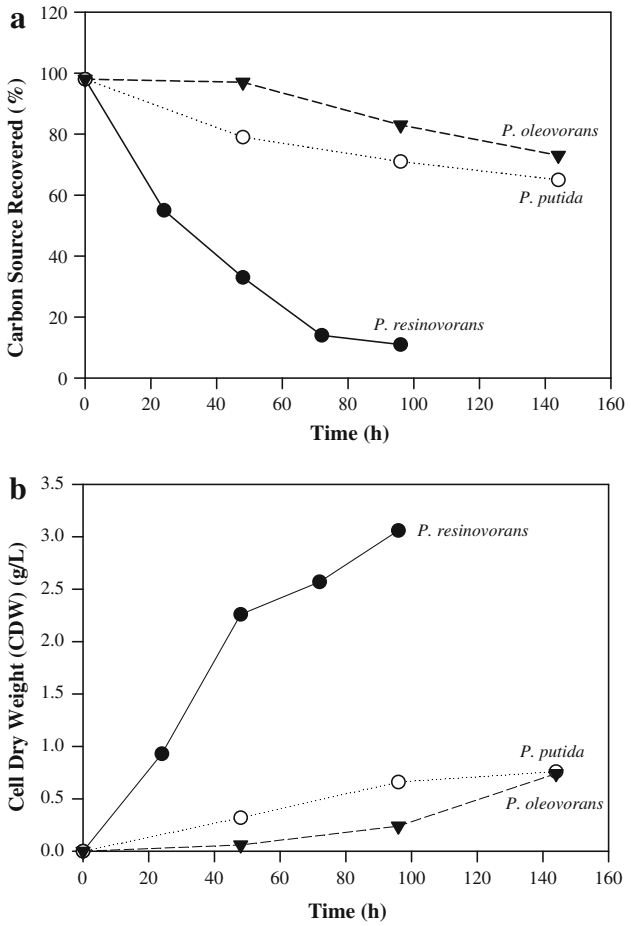


Fig. 1 Carbon source recovered **a** and cell dry weights **b** from the cultures containing *P. resinovorans*, *P. putida* and *P. oleovorans* as a function of time

In order to monitor preferential, carbon source utilization and degradation products, the recovered carbon source from each of the specific bacterial strains was analyzed by GC and GC–MS. Table 1 shows the results of those analyses. Based on those results, it was concluded that sbc–FA can be effectively degraded by each of the bacterial strains tested albeit to varying degrees. Table 1, entries 2–9 list the sbc–FA product distribution in the recovered carbon source after incubation for 48 h–144 h with each of the bacterial strains. The results showed that the sbc–FA mixture in particular, the isostearic acid was selectively utilized by each of the bacterial strains. For example, in the presence of *P. resinovorans* at 30 °C for 48 h, the concentration of isostearic acid was reduced by 17 % from 75.4 wt% to 62.4 wt% (Table 1, entry 2). Prolonging the incubation time to 96 h led to a further significant decrease of isostearic acid to 48 wt% yield (a total decrease of 36 %; Table 1, entry 3). *P. putida* and *P. oleovorans* were also capable of utilizing the isostearic acid fraction (Table 1, entries 4–9). Interestingly, for all three strains the percentage of isostearic acid steadily dropped initially, but leveled off at ~40 % after 144 h. Although, based on cellular growth and carbon source recovery, all three strains could degrade isostearic acid, it was determined that *P. resinovorans* was actually more equipped to utilize isostearic acid at a faster rate. Thus subsequent work focused on this strain. As shown in Table 2, the time-dependent degradation studies clearly indicated that *P. resinovorans* degraded isostearic acid from 72.4 wt% to 39.3 wt% (entries 1–7) after it was incubated for 168 h. These results imply that the isostearic acid was only

partially utilized by *P. resinovorans*, under the conditions tested. Beta-oxidation is the most well known means by which fatty acids are broken-down; however, 3-methyl-substituted fatty acids cannot undergo β-oxidation, because the 3-methyl-group prevents the formation of the 3-keto substituent in the dehydrogenation step, which impedes further β-oxidation. In the present study, the isostearic acid carbon source was a mixture, where the methyl group was located at different positions along the carbon backbone, thus it was probable that some β-oxidation did occur initially, but as the fatty acids were shortened and converted to 3-methyl-branched derivatives, β-oxidation stopped leaving approximately 40 wt% of the starting isostearic acids as unutilized at 96 h and above. The sbc–FA mixture used as carbon source in this study (Table 2, entry 1) was slightly different from the material reported in Table 1, mainly due to experimental variation in sample preparation and data analysis. For all subsequent studies, two important points should be noted: first, the bacterial strains also degraded the stearic acid present in the carbon mixture, but to a much smaller extent than the isostearic acid; second, when the percentages of isostearic acid and stearic acid decreased due to degradation, the percentages of γ-stearolactones (Scheme 1, [**4**] and [**5**]) and dimer products (Scheme 1, [**6**]) which were not utilized by the bacterial strains showed an increase. It was not, because the bacterial strains produced more of these materials.

From the GC and GC–MS results, we identified the new materials generated by *P. putida* and *P. oleovorans* (Table 1, entries 4–9), but were having difficulty identifying the new materials generated by *P. resinovorans* (Table 1, entries 2, 3). For instance, Fig 2 shows the GC chromatogram of the recovered crude sbc–FA from *P. putida* after methylation (data for recovered crude carbon mixture from *P. oleovorans* was comparable to that from *P. putida*, so is not shown). The mass spectral analysis showed 4 new types of fatty acids being formed. The peaks labeled A are consistent with C_{10}-branched-chain FAME isomers with $M^{+\bullet}$ at *m/z* 186, B: C_{12}-branched-chain FAME isomers with $M^{+\bullet}$ at *m/z* 214, D: C_{14}-branched-chain FAME isomers $M^{+\bullet}$ at *m/z* 242, and E: C_{16}-branched-chain FAME isomers $M^{+\bullet}$ at *m/z* 270. These results were compared against a standard which contained C10–C18 saturated linear chain FAME (data not shown). The retention times for the linear chain FAME versus branched-chain FAME of various chain lengths were very similar. These results further supported, we had generated C_{10}, C_{12}, C_{14} and C_{16}-branched-chain fatty acid isomers during the incubation process. In addition, we suggested that these were short chain fatty acid isomers, because the molecular ions obtained by the MS were the same. For example, the mass spectra of the peaks within group B of Fig. 2 were different, but they all showed the same molecular ion, indicating their isomeric nature Fig 3. In addition, fragment *m/z* 183 corresponds to the loss of $CH_3O\bullet$ (M-31), which is expected for FAME products and the ion at *m/z* 74 is a result of a rearrangement of the fatty acid known as the McLafferty rearrangement. The fragmentations further supported that the new materials formed during degradation are short chain FAME isomeric products. On the other hand, the new group of products generated by *P. resinovorans* observed eluting with a retention time of approximately 19.3 min for the more abundant constituent, and representing between 4 % and 10 % of the chromatographic profile (Fig. 4, B) was found to be quite challenging to identify. These products were observed for methylated samples only with very similar spectra, showing a high mass ion at *m/z* 250, and an ion series

Table 2 Carbon source content of the recovered saturated branched chain-FA after exposure to *P. resinovorans* for various times[a]

Entry	Bacterial strain	Time [h]	Percentage composition from GC				
			Methyl isostearates [**2**[b]]	Methyl stearate [**3**[b]]	γ-Stearolactones [**4**[b] & **5**[b]]	C_{36}-dimer FAME [**6**[b]]	Unknowns
1	None	0	72.4	3.2	13.2	11.2	0
2	*P. resinovorans*	24	72.2	3.0	10.8	14.0	0
3	*P. resinovorans*	48	63.5	3.4	14.3	16.6	2.2
4	*P. resinovorans*	72	48.1	4.1	16.4	20.3	11.1
5	*P. resinovorans*	96	41.2	4.6	20.3	21.0	12.9
6	*P. resinovorans*	120	39.2	4.5	18.6	25.8	11.9
7	*P. resinovorans*	168	39.3	5.3	18.4	26.8	10.2

FAME fatty acid methyl esters, *FA* fatty acids, *P Pseudomonas*

[a] Starting fatty acids are sbc–FA. Products were methylated and then analyzed by GC. Results were compared against internal standard methyl tridecanoate (C13:0)

[b] Referring to Scheme 1

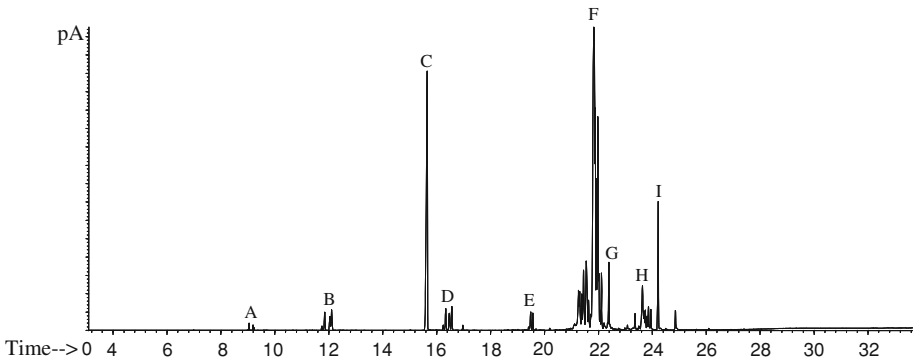


Fig. 2 GC-FID chromatogram of the methylated sbc–FA recovered from *P. putida*. C_{10} methyl-branched-chain FAME isomers (*A*), C_{12} methyl-branched-chain FAME isomers (*B*), $C_{13:0}$ internal standard (methyl tridecanoate; *C*), C_{14} methyl-branched-chain FAME isomers (*D*), C_{16} methyl-branched-chain FAME isomers (*E*), C_{18} methyl-branched-chain FAME isomers (methyl isostearates; *F*), C_{18} linear chain FAME (methyl stearate; *G*), branched-chain-γ-stearolactones (*H*), linear chain-γ-stearolactone (*I*). *FA* fatty acids, *FAME* fatty acid methyl ester

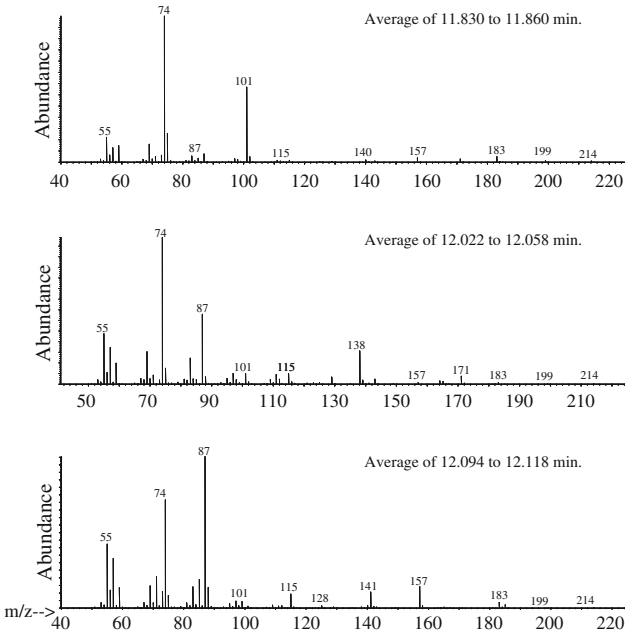


Fig. 3 Mass spectral traces of the saturated C_{12}-branched-chain fatty acid methyl ester isomers from *B* in Fig. 2

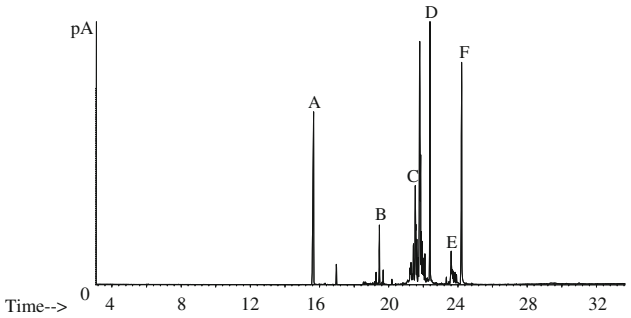


Fig. 4 GC chromatogram of methylated sbc–FA recovered from *P. resinovorans*. $C_{13:0}$ internal standard (methyl tridecanoate; *A*), unknowns (*B*), C_{18} methyl-branched-chain FAME isomers (methyl isostearates; *C*), C_{18} linear chain FAME (methyl stearate; *D*), branched-chain-γ-stearolactones (*E*), linear chain-γ-stearolactone (*F*). *FA* fatty acids, *FAME* fatty acid methyl esters

characteristic of an alkyl chain at 14 Da apart and the base peak at *m*/*z* 67 or 95. The retention time and the spectrum of these products were not altered by silylation (i.e., addition of trimethylsilyl to hydroxyl group), showing the absence of a hydroxyl group in the structure. The fact that they were methylated products indicated carboxylic acid functionality associated with an alkyl chain, but the low-concentration and the lack of diagnostic ions in the high mass region of the spectra made it difficult to characterize their structure. These products are reported as unknowns in Tables 1 and 2 and currently cannot be recognized as degradation products from the carbon source or the dead bacterial cells.

In an attempt to identify further the materials generated by the bacterial strains, control experiments were carried out with the unsaturated form of the branched-chain fatty acid mixture (ubc-FA). Table 3 lists the results of the ubc-FA incubated with *P. resinovorans* under similar conditions as the sbc–FA mixtures. The fatty acids were recovered, hydrogenated and methylated to facilitate the analysis. The results showed that *P. resinovorans* utilized the unsaturated fatty acid mixture in a similar fashion to the sbc–FA mixture. Interestingly, the new fatty acids generated by *P. resinovorans* (which was approximately 10 % after incubation for 192 h) using ubc-FA were similar to the ones produced by *P. putida* and *P. oleovorans* from sbc–FA. The GC chromatogram of the recovered ubc-FA oils after hydrogenation and methylation also showed the formation of 4 new types of fatty acids with molecular ions

Table 3 Carbon source content of the recovered saturated branched chain-FA after exposure to *P. resinovorans* for various times[a]

Entry	Bacterial strain	Time [h]	Percentage composition from GC				
			Methyl isostearates [**2**[b]]	Methyl stearate [**3**[b]]	γ-Stearolactones [**4**[b] & **5**[b]]	C_{36}-dimer FAME [**6**[b]]	C_{10},C_{12},C_{14},C_{16}-branched FAME
1	None	0	74.6	7.8	8.9	8.8	0
2	*P. resinovorans*	24	72.7	6.5	7.1	9.4	4.3
3	*P. resinovorans*	48	67.4	3.3	10.4	11.4	7.6
4	*P. resinovorans*	72	61.7	2.3	9.6	12.9	13.5
5	*P. resinovorans*	96	57.2	1.4	14.3	14.0	13.1
6	*P. resinovorans*	168	55.7	2.4	16.4	16.0	9.5
7	*P. resinovorans*	192	53.9	1.6	16.5	18.1	9.9

FAME fatty acid methyl esters, *FA* fatty acids, *P Pseudomonas*

[a] Starting fatty acids are sbc–FA. Products were methylated and then analyzed by GC. Results were compared against internal standard methyl tridecanoate (C13:0)

[b] Referring to Scheme 1

corresponding to C_{10}, C_{12}, C_{14}, and C_{16}-branched-chain fatty acid isomers (Table 3). Unfortunately, this information still did not help us identify the fatty acids generated by *P. resinovorans* in the sbc–FA system.

Although, we were unable to definitively identify all the breakdown products from *P. resinovorans* in the sbc–FA system, we were able to show that *P. resinovorans*, *P. putida* and *P. oleovorans* do have the capability of biodegrading the isostearic acid fraction of the sbc–FA mixtures used in this study. *P. resinovorans* demonstrated the fastest isostearic acid degradation, thus subsequent work will focus on this strain. By demonstrating the degradable nature of these sbc–FA, it is envisioned that these materials will be aided in their potential for "environmentally friendly" applications.

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