Biohydrogenation of Linoleic Acid by Anaerobic Bacteria Isolated from Rumen

Kenshiro FUJIMOTO, Hiromi KIMOTO, Mieko SHISHIKURA, Yasushi ENDO, and Keiji OGIMOTO

Faculty of Agriculture, Tohoku University, 1-1, Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981, Japan

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The hydrogenation of unsaturated oils is an important chemical process for modifying their physical and chemical properties, such as melting point and oxidative stability, in the oil industry. However, this reaction requires high temperature, high pressure, and metallic catalysts while it gives many trans isomers as by-products, which are unfamiliar in natural oils. Recently, biosynthesis and biotransformation of unique fats and fatty acids using microorganisms have been developed. These processes are superior to the chemical ones in their substrate and product specificity, and are done under much milder conditions than the chemical modification.

It is well known that most dietary unsaturated fatty acids in the rumen of ruminant animals are hydrogenated by microorganisms. Some rumen bacteria capable of hydrogenating unsaturated fatty acid such as linoleic and linolenic acids have been identified as Butyviribrio fibrisolvens, Micrococcus sp., Treponema, Ruminococcus sp., Eubacterium sp., Clostridium, Bacteroides sp., and Propionibacterium.

In this study, anaerobic bacteria isolated from the rumen of sheep were screened for their hydrogenation ability to get bacteria applicable for pure culture biohydrogenation of unsaturated fats and oils.

Linoleic acid was purchased from Sigma Chemical Co. (St. Louis, U.S.A.).

Rumen fluid was collected from two female merino sheep which had been fed with commercial alfalfa hay cubes and concentrates supplemented with fish oil or phytosterol for 8 weeks. The roll tube method was applied for the isolation of the rumen bacteria and the screening tests. At first, rumen bacteria capable of digesting cellulose were isolated, using a cellulose medium, because most cellulose-digesting bacteria have been shown to have hydrogenation ability. After incubation of the isolated cellulose-digesting bacteria in 10 ml of yeast Extract-Trypticase-Rumen Fluid (YTR) broth at 37°C for 24 h, the bacteria were collected by anaerobic centrifugation. They were then anaerobically incubated in YTR broth containing linoleic acid (0.5 mg/ml) at 37°C for 24 h. The control was without the added linoleic acid. Free fatty acids were extracted from the supernatant of the broths with diethyl ether after centrifugation. The fatty acid composition was analyzed by gas liquid chromatography (GLC) using a CP-Si188 capillary glass column (0.25 mm × 50 m, Chrompack Co., Holland) after methylation with BF₃-methanol. The position of the double bond on the monoenoic acids was identified with a JEOL JMS HX-105 gas chromatograph-mass spectrometer (GC-MS) using an HT-5 stainless column (0.53 mm × 12 m, Chrompack) by the method of Shibahara et al.

The identification of rumen bacteria was made by the Gram stain, biochemical tests, the analysis of the generated volatile fatty acids, and morphological features.

Ninety strains of cellulose-digesting bacteria were screened to assess their hydrogenation ability. As shown in Table 61 strains of anaerobic bacteria capable of hydrogenating linoleic acid were found. They hydrogenated more than 50% of added linoleic acid. The anaerobic rumen bacteria with hydrogenation ability were divided into three groups based on the varieties of hydrogenation products of the linoleic acid. Fifty-seven strains hydrogenated linoleic acid to the trans-11-octadecenoic acid (trans-11-18:1), and were identified as Butyviribrio fibrisolvens, which is known to have hydrogenation capability. On the other hand, two strains of unidentified bacteria, which produced succinate and propionate as terminal products of glucose metabolism, hydrogenated the linoleic acid not only to trans-11-18:1 but also to stearic acid (18:0). trans-9-Octadecenoic acid (trans-9-18:1) was found in the other two strains (Group III). One of these was identified as Butyviribrio fibrisolvens, and the other was Selenomonas ruminantium. Butyviribrio fibrisolvens A38 has been reported as a bacterium producing trans-9-18:1.

In this study, Selenomonas ruminantium was identified as a hydrogenating bacterium for the first time, although it was previously recognized as a lipolytic bacterium.

Based on these results, we discuss the possible pathways of the hydrogenation of linoleic acid by rumen bacteria. Two types of hydrogenation pathways are possible at least, as shown in the scheme. Most of the rumen bacteria with hydrogenation ability (ex. Butyviribrio fibrisolvens) isomerize the linoleic acid (cis-9, 12-octadecadienoic acid) to the cis-9, trans-11-octadecadienoic acid (cis-9, trans-11-18:2) by an isomerase in the first step. The double bond at position 9 in the conjugated octadecadienoic acid is preferentially reduced to form trans-11-18:1. Two of the rumen bacteria can also reduce the trans-11-18:1 to stearic acid. This pathway has been already recognized by some researchers, and we also detected cis-9, trans-11-18:2 in linoleic acid biohydrogenated by some Butyviribrio fibrisolvens strains of rumen bacteria. A pathway to produce trans-9-18:1 has not been described. However, it is assumed that a Selenomonas ruminantium and one of Butyviribrio fibrisolvens isolates probably could isomerize the linoleic acid to the trans-9, trans-11-octadecadienoic acid (trans-9, trans-11-18:2), via the cis-9, trans-11 isomer, since trans-9, trans-11-18:2 has been also found in linoleic acid biohydrogenated by Butyviribrio fibrisolvens. They preferentially could reduce the double bond at position 11 of conjugated octadecadienoic acid to produce trans-9-18:1. As a result, all the rumen bacteria with a hydrogenation ability found in this study could produce the trans type of monoenoic acid from linoleic acid, but not the cis type.

We also screened rumen bacteria for the hydrogenation of trilinolein, linolenic acid (18:3, n-3), and eicosapentaenoic acid.

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<th>Biohydrogenation of Linoleic Acid by Rumen Bacteria</th>
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<tr>
<td>Bacterium group</td>
<td>Hydrogenation products</td>
</tr>
<tr>
<td>I</td>
<td>trans-11-18:1</td>
</tr>
<tr>
<td>II</td>
<td>trans-11-18:1 + 18:0</td>
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<td>III</td>
<td>trans-9-18:1</td>
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Note
Bihydrogenation of Linoleic Acid by Rumen Bacteria

**Scheme.** Possible Biohydrogenation Pathways of Linoleic Acid by Rumen Bacteria.

(20:5, EPA). (Data not shown.) Although all the bacteria capable of hydrogenating the linoleic acid hydrogenated the linolenic acid, none of the isolates hydrogenated trilinolein or EPA. These observations suggest that hydrogenation by rumen bacteria is effective only for free unsaturated fatty acids. As to the biohydrogenation of EPA, some modification of the assay method will be necessary, because the growth of bacteria was seriously retarded by EPA, the antimicrobial activity of which has been reported.

References