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Analysis of Peculiar Fatty Acid Transformation by Anaerobic Microorganisms and Application to Conjugated Fatty Acid Production

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Research aims

Various fatty acids with conjugated double bonds occur in nature. For example, edible fats derived from ruminant animals contain conjugated linoleic acid (CLA), which mainly consists of *cis*-9,*trans*-11- and *trans*-10,*cis*-12-octadecadienoic acid (18 : 2). The occurrence of conjugated fatty acids has also been reported in plants, for example, *cis*-9,*trans*-11,*trans*-13-octadecatrienoic acid (18 : 3) in *Momordica charantia* seed oil. The secondary metabolism of fatty acids by marine algae involves polyunsaturated fatty acids (PUFAs) containing conjugated olefin systems, for example, *cis*-5,*trans*-7,*trans*-9,*cis*-14-eicosatetraenoic acid (20 : 4) produced from arachidonic acid (*cis*-5,*cis*-8,*cis*-11,*cis*-14-20 : 4). These conjugated fatty acids have attracted much attention as a novel type of biologically beneficial functional lipid[1–3]. In particular, the unique activities of CLA have been intensively studied, and there are many reports that CLA reduces carcinogenesis, atherosclerosis, and body fat[4,5]. So, conjugated fatty acids are expected to be potential materials for pharmaceuticals and dietary supplements.

In this study, I examined whether *Clostridium bifermentans*, which was previously shown to have the ability to produce CLA from linoleic acid, can transform various PUFAs into conjugated fatty acids. The results demonstrated that a variety of conjugated fatty acids could be produced from PUFAs by the action of *C. bifermentans*. A putative reaction pathway for the observed fatty acid transformation was proposed based on the results obtained.

Methods

Clostridium bifermentans was inoculated into GAM medium and cultured for 3 days. Each transformation reaction was performed at 37°C in a 1.0 ml of reaction mixture containing 0.6 mg the fatty acid (as the substrate), washed cells and 0.1 M potassium phosphate buffer (pH 6.5). After reactions, lipid extraction and methyl esterification were carried out and fatty acid methyl esters were then separated by gas chromatography for analysis and by high-performance liquid chromatography for purification. Structures of purified methyl esters were identified by NMR and MS analysis.

Results

In order to examine substrate specificity of the reactions catalyzed by washed cells of *Clostridium bifermentans*, a number of unsaturated fatty acids were subjected as the substrate in the reaction. The results were summarized at Table 1. A wide range of C_{18} and C_{20} unsaturated fatty acids

Table 1. Production of conjugated fatty acids by *C. bifermentans*

C18 FAs	Substrate	Products
Linoleic acid	(9*Z*,12*Z*-18:2)	(9*Z*,11*E*-18:2, 9*E*,11*E*-18:2)
α-Linolenic acid	(9*Z*,12*Z*,15*Z*-18:3)	(9*Z*,11*E*,15*Z*-18:3, 9*E*,11*E*,15*Z*-18:3)
γ-Linolenic acid	(6*Z*,9*Z*,12*Z*-18:3)	(6*Z*,9*Z*,11*E*-18:3, 6*Z*,9*E*,11*E*-18:3)
Columbinic acid	(5*E*,9*Z*,12*Z*-18:3)	(5*E*,9*Z*,11*E*-18:3, 5*E*,9*E*,11*E*-18:3)
Stearidonic acid	(6*Z*,9*Z*,12*Z*,15*Z*-18:4)	(6*Z*,9*Z*,11*E*,15*Z*-18:4, 6*Z*,9*E*,11*E*,15*Z*-18:4)
C20 FAs	**Substrate**	**Products**
Eicosadienoic acid	(11*Z*,14*Z*-20:2)	(11*Z*,13*E*-20:2, 11*E*,13*E*-20:2)
Eicosatrienoic acid	(11*Z*,14*Z*,17*Z*-20:3)	(11*Z*,13*E*,17*Z*-20:3, 11*E*,13*E*,17*Z*-20:3)
Dihomo-γ-linolenic acid	(8*Z*,11*Z*,14*Z*-20:3)	(8*Z*,11*Z*,13*E*-20:3, 8*Z*,11*E*,13*E*-20:3)
ω-3 Arachidonic acid	(8*Z*,11*Z*,14*Z*,17*Z*-20:4)	(8*Z*,11*Z*,13*E*,17*Z*-20:4, 8*Z*,11*E*,13*E*,17*Z*-20:4)
Arachidonic acid	(5*Z*,8*Z*,11*Z*,14*Z*-20:4)	(5*Z*,8*Z*,11*Z*,13*E*-20:4, 5*Z*,8*Z*,11*E*,13*E*-20:4)
Eicasapentaenoic acid	(5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-20:4)	(5*Z*,8*Z*,11*Z*,13*E*,17*Z*-20:5, 5*Z*,8*Z*,11*E*,13*E*,17*Z*-20:5)

Z, *cis* configuration; *E*, *trans* configuration

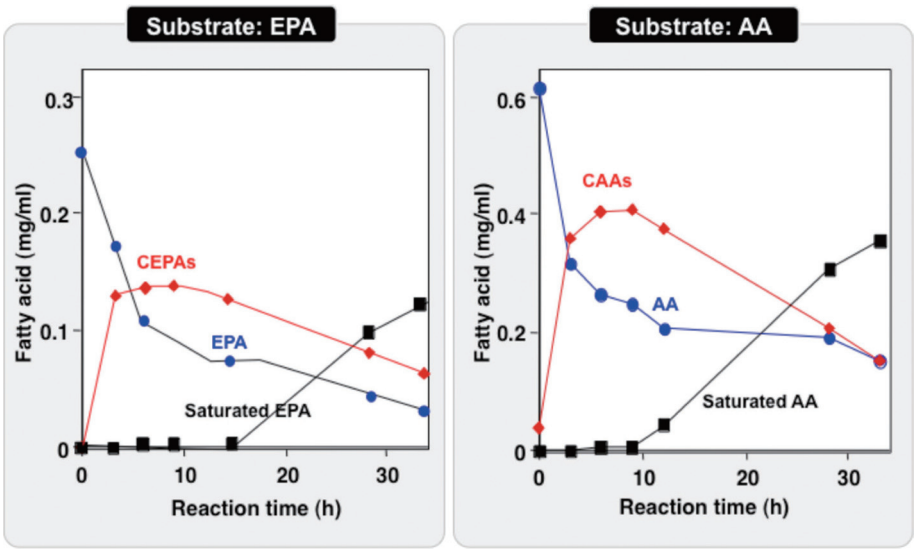
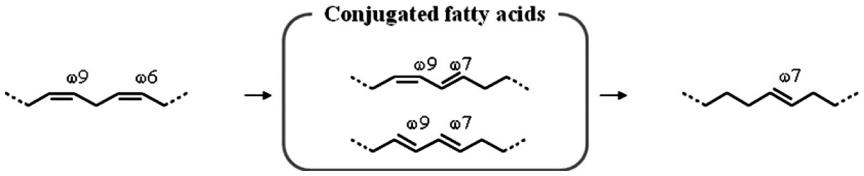


Fig. 1. Time course of EPA and AA transformation by *C. bifermentans.*



Scheme 1. Putative pathway of polyunsaturated fatty acid transformation by *C. bifermentans*.

could be reacted with cells of *C. bifermentans* and were converted to their respective conjugated isomers. Polyunsaturated fatty acids that could be substrates for the transformation reaction mediated by *C. bifermentans* included α-linolenic acid, γ-linolenic acid, columbinic acid, stearidonic acid, eicosadienoic acid, eicosatrienoic acid, dihomo-γ-linolenic acid, ω3 arachidonic acid, arachidonic acid (AA), and eicosapentaenoic acid (EPA) with *cis* double bonds at ω6 and ω9 position.

When cells of *C. bifermentans* were incubated with linoleic acid, AA, or EPA for longer periods of time, additional fatty acids were produced concomitantly with the generation of conjugated fatty acids. These fatty acids were identified as *trans*-11-octadecenoic acid from linoleic acid, *cis*-5,*cis*-8,*trans*-13-eicosatrienoic acid from AA, and *cis*-5,*cis*-8,*trans*-13,*cis*-17-eicosatetraenoic acid from EPA. The time courses of EPA and AA conversion were monitored (Fig. 1). EPA and AA were initially converted to conjugated EPA (CEPAs) and conjugated AA (CAAs), respectively. When the reactions were allowed to proceed for longer time, the amounts of CEPAs and CAAs were decreased with the concomitant increase in the amounts of saturated EPA and saturated AA, respectively (Fig. 1).

These results suggested that this strain recognizes ω6 and ω9 double bonds in the substrate fatty acids and rapidly converts *cis*-ω6,*cis*-ω9 to *trans*-ω7,*cis*-ω9 and *trans*-ω7,*trans*-ω9, and finally reduces the double bond at ω9 position in conjugated fatty acid (Scheme 1).

References

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