Occurrence of Conjugated Dienoic Fatty Acids in the Cellular Lipids of *Pediococcus homari*

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It has been generally believed that bacteria are unable to synthesize polyunsaturated fatty acids, in contrast to all other life forms.¹⁾ Only one exception hitherto unequivocally known is *Bacillus licheniformis* ATCC 9295²⁾ which was shown to form a small amount of 5,10-hexadecadienoic acid through repeated oxidative desaturations of palmitic acid, under the limited conditions of cultural temperature. The present communication describes the occurrence of several conjugated di-unsaturated fatty acids in the cellular lipids of *P. homari*, a moderately halo-tolerant lactic acid coccus.

Previously, the author found an appreciable amount of unknown fatty acids with unusual GLC properties in the cellular fatty acids of

P. homari.³⁾ A preliminary examination of these unknown acids and their hydrogenated products on GLC columns of different polarities suggested that they were relatively highly unsaturated fatty acids of C₁₆, C₁₈, and C₂₀.⁴⁾

P. homari NISL 7136 was cultivated in the medium A as previously reported.⁴⁾ Conditions for harvest of the cells and for extraction, methylation and GLC analysis of the cellular fatty acids were almost the same as described before.^{4,5)} The methyl esters were then separated into saturated, mono-unsaturated and slow migrating unknown fatty acid fractions by means of AgNO₃-impregnated silicagel thin-layer chromatography.⁶⁾ When the unknown fraction was analyzed by GLC, four major peaks were obtained along with several minor components. Hydrogenation of the unknown fraction under 1 atm with a palladium catalyst resulted in the production of palmitic and stearic acids, suggesting that the unknown acids were consisted mainly of C₁₆-, and C₁₈-straight-chain unsaturated acids.

An extensive examination of GLC behavior of the unknown acids with DEGS (diethyleneglycol succinate polyester, 20% on Chromosorb W DMCS, 3 mm × 2 m), XE-60

FIG. 1. Mass Spectrum of the Methyl *cis,cis*-Conjugated Hexadecadienoate Isolated from *P. homari*.

Mass spectrum was obtained from a Hitachi 063-RMU-7M combined GLC-mass spectrometer working with an ionizing electron energy of 70 eV, an accelerating voltage of 3.2 kV, and an electron current of 100 μ A. The separator and the connection part were maintained at 250°C. The GLC column was a 3 mm × 2 m stainless steel column packed with 10% FFAP/Diasolid L, 60~80 mesh, and operated at 200°C with He as the carrier gas.

FIG. 2. Ultraviolet Absorption Spectrum of the Methyl *cis,cis*-Conjugated Hexadecadienoate Isolated from *P. homari*.

About 8 $\mu\text{g/ml}$ solution of the methyl ester in spectral grade *n*-hexane (Tokyo Kasei Kogyo Co., Ltd.) was determined for its UV absorption with a Hitachi 323 Recording Spectrophotometer.

(15%, 3 mm $\times$ 1 m), and SE-52 (10%, 3 mm $\times$ 1 m) columns revealed that the major acids could be grouped into two homologous series, X or Y, mutually distinguishable but similar in structure. Thus, the four major unknown acids having equivalent chain lengths of 18.14, 18.44, 20.14, and 20.50 on the DEGS column were temporarily designated as 16-X, 16-Y, 18-X, and 18-Y, respectively. The number denotes the length of the carbon chain, and X or Y indicates the homologous series of the acids.

Gas chromatography-mass spectrometry showed that the four unknown acids were all di-unsaturated acids⁷⁾ (Fig. 1). Unexpectedly, the mass spectra of X-, and Y-acids of the same chain lengths were almost consistent with each other. This suggested that the X-, and Y-series acids differed only in the configuration of the unsaturated bonds.

The unknown acids were separated individually by preparative GLC⁶⁾ using a 3 mm $\times$ 2 m column packed with 15% SE-52 coated Chromosorb W DMCS 60~80 mesh. Four isolates of 80~85% purities, 16-X, 16-Y, 18-X, and 18-Y, were obtained and were determined for their ultraviolet and infrared spectra.⁶⁾ All these unknown acids were

shown to have strong ultraviolet absorption (ϵ =about 2.8×10^4), characteristic to conjugated dienes. The absorption maxima of 16-X and 18-X were at 233 nm, and those of 16-Y and 18-Y were at 235 nm; this finding suggested that the X-, and Y-series acids had *cis,trans*-, and *cis,cis*-configurations,⁸⁾ respectively. An infrared spectrum of 16-X showed two absorption bands at 948 and 982 cm^{-1} ; these facts were indicative of a *cis,trans*-conjugated dienoic structure.⁸⁾ Spectra of both 16-Y and 18-Y, however, did not show any diagnostic absorption over the wave range of 900~1000 cm^{-1} , indicating that the conjugated dienes in the Y-series acids were *cis,cis*-configuration. Infrared spectrometry of 18-X was not performed because of the meager amount of the isolate.

On the bases of these experimental data, the 16-X, 16-Y, 18-X, and 18-Y acids were tentatively identified as *cis,trans*-conjugated hexadecadienoic, *cis,cis*-conjugated hexadecadienoic, *cis,trans*-conjugated octadecadienoic, and *cis,cis*-conjugated octadecadienoic acids, respectively. The retention data of C_{18} -conjugated dienoic acids in GLC are almost consistent with those published before.⁹⁾ Determination of the positions of conjugated double bonds in these unusual acids is now in progress. It was assumed, however, from the mass spectral data, that the double bonds were located relatively closely to the carboxyl groups in the C_{16} -acids.⁷⁾

To our best knowledge, this is the first report for isolation of conjugated dienoic acids from bacteria.¹⁰⁾ It should be noted that these unusual acids comprised up to 80% of the total fatty acids in this bacterium, under certain cultural conditions. A new mechanism for synthesizing unsaturated fatty acid must be involved in the fatty acid biosynthesis by *P. homari*.

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