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Fatty Acid Composition of *Vibrio vulnificus*, *Escherichia coli* and *Salmonella typhimurium* Lipopolysaccharide(LPS)

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Vibrio vulnificus, *Escherichia coli* 및 *Salmonella typhimurium* Lipopolysaccharide(LPS)의 지방산 조성

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ABSTRACT

*Vibrio vulnificus*에서 lipopolysaccharide(LPS)를 추출하여 지방산 조성을 분석한 후 이 결과를 *Escherichia coli* LPS와 *Salmonella typhimurium* LPS의 것들과 비교하였다. *Vibrio vulnificus* LPS의 주 지방산은 myristic acid(C14 : 0, 41.37%)이었고 *Escherichia coli* LPS는 lauric acid(C12 : 0, 37.03%), *Salmonella typhimurium* LPS는 capric acid(C10 : 0, 48.60%)로 서로 달랐으나 이 세가지 지방산이 각 LPS의 주성분이었다(70% 이상).

I. Introduction

Lipopolysaccharide(LPS), endotoxin, is an integral part of the cell wall of gram-negative bacteria,[1] and is known to have broad spectra of biochemical and immunobiological activities.[2] LPS from Enterobacteriaceae bacteria consists of a polysaccharide with repeating units(0 antigen), an oligosaccharide(core antigen), and a glycolipid(lipid A).[3] The extraction of lipid A from LPS is done by hydrolysis.

Lipid A contains a central backbone of the phosphorylated $\beta(1->6)$ linked glucosamine disaccharide. Attached to the disaccharide are a variety of esterified and amidated fatty acids.[4] For almost all of gram-negative bacteria, the main fatty acids are known to be lauric acid, myristic acid, and palmitic acid.

The halophilic bacterium *Vibrio vulnificus* causes acute, fulminating wound infections, and septicema in humans.[5] These infectious diseases have evoked a national sensation in Korea not only on the societies of microbiology, immunology, and medical science, but also on the viewpoint of the protection of people against infection. We, as biochemists, are eager to know the lethality of the host, but we know little.

Although the problem of the role of LPS in infection, has been a difficult one to approach experimentally, we hope that our study may be used to determine LPS's roles in virulence of *V.*

vulnificus infection, identify it, and may form a sound basis for the development of vaccine.

Therefore, in this report *V. vulnificus*, *E. coli*, and *S. typhimurium* LPSs were isolated by the hot phenol-water method and the fatty acid compositions of the LPSs were analyzed.

Ⅱ. Materials and Methods

1. Bacteria and growth conditions

The bacterial strains in this study were *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14208, and *Vibrio vulnificus* P-1. *E coli* and *S. typhimurium* were grown with vigorous aeration in TSB at 37℃ for 24h. A virulent strain of *V. vulnificus* was grown in TSB which contained additional 5g of NaCl.

2. Extraction and purification of LPS

The hot phenol-water procedure of Westphal was used to extract and purify the LPSs from *E. coli*, *S. typhimurium* and *V. vulnificus*.[6] Acetone dried cells were suspended in 100ml of distilled water, sonicated for 3 min with cooling, and centrifuged at 4,000g for 1h. The resulting precipitate was suspended in 50ml of distilled water, sonicated, and added 50ml of 90% phenol. The mixture was homogenized at 65~68℃ for 20 min, cooled to 10℃ in ice bath, and centrifuged at 4,000g for 1h. The upper solution(the aqueous phase) was removed and the lower solution(the phenol phase and insoluble precipitate) was reextracted with 50ml of distilled water. The combined solution of upper solution was dialyzed against distilled water and a half volume of $CHCl_3$-BuOH solution(5 : 1, v/v) was added. The mixture was centrifuged at 3,000g for 10 min. The upper solution was removed and a tenth volume of 2% hexadecyl trimethyl ammonium bromide was treated. The solution was homogenized for 20 min and centrifuged at 4,000g for 20 min. The supernatant was dialyzed against distilled water freeze-dried.

3. Fatty acid analysis of LPS

Fatty acids were analyzed as their methyl esters by GC.[7] For fatty acid analysis, LPS(2 mg) was hydrolyzed in 2 N HCl. The hydrolysate was suspended in chloroform, methanol, and water solution(4 : 10 : 5, v/v). The mixture was centrifuged at 5,000g for 20 min. The chloroform phase was dried by evaporation and acidified with 2 N HCl. 10% BF_3-MeOH was added to catalyze the methylation. Gently mix and heat in a water bath at 100℃ then cool to room temperature. n-Hexane was added to extract the fatty acids methyl esters from the aqueous phase. Tightly seal the tube and gently but thoroughly mix the tube. Allow a few minutes for the phases to separate, pipette and discard the acidified aqueous phase(bottom phase). Add 0.3 N NaOH solution to the organic extract. Cap gently and mix. When the phases are separated, transfer the organic extract(top phase) to a clean sample vial. Quantitate in a GC, fitted with glass colum (3mm by 3m) of 10% 1,4-butanediol succinate on Chromosorb W(60~80 mesh) at 185℃.

Ⅲ. Results and Discussions

Fatty acid composition of LPS

The gas chromatograms of the fatty acids methyl esters of standard fatty acids, *E. coli*. *S. typhimurium*, and *V. vulnificus* LPSs were shown in Fig. 1, 2, 3, and 4.

The fatty acid compositions of LPSs were summarized in Table 1. The most abundant fatty acid was myristic acid(C14 : 0) for *V. vulnificus* LPS, lauric acid(C12 : 0) for *E. coli* LPS, and capric acid(C10 : 0) for *S. typhimurium* LPS. But these three fatty acids were the major components for three LPSs(more than 70%). The

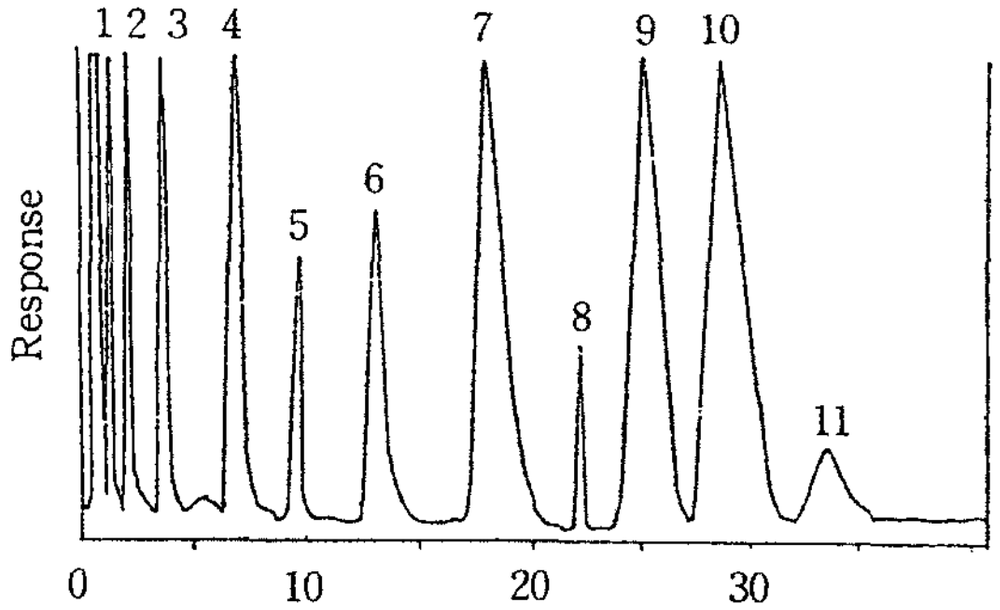

Fig. 1. GC of bacterial fatty acids methyl esters standard mixture.

1. C 8 : 0, 2. C10 : 0, 3. C12 : 0,
4. C14 : 0, 5. C16 : 0, 6. C17 : 0,
7. C18 : 0, 8. C18 : 1, 9. C18 : 2,
10. C18 : 3, 11. C20 : 0.

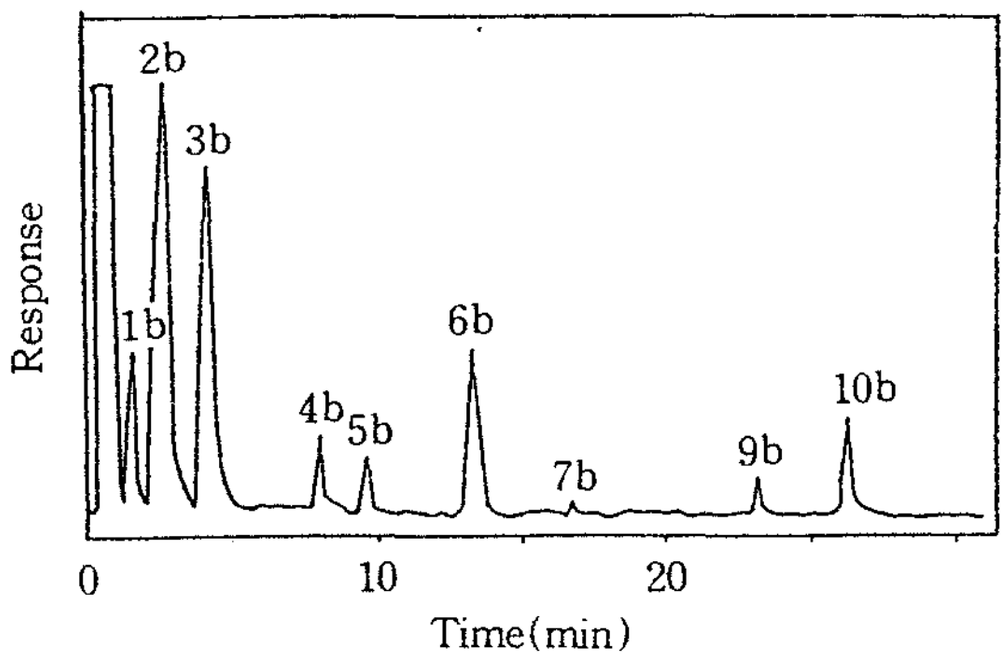

Fig. 3. GC of methyl esters of fatty acids from S. typhimurium LPS.

Peaks designated as 1b to 7b, 9b, and 10b are the corresponding peaks in the Fig. 1.

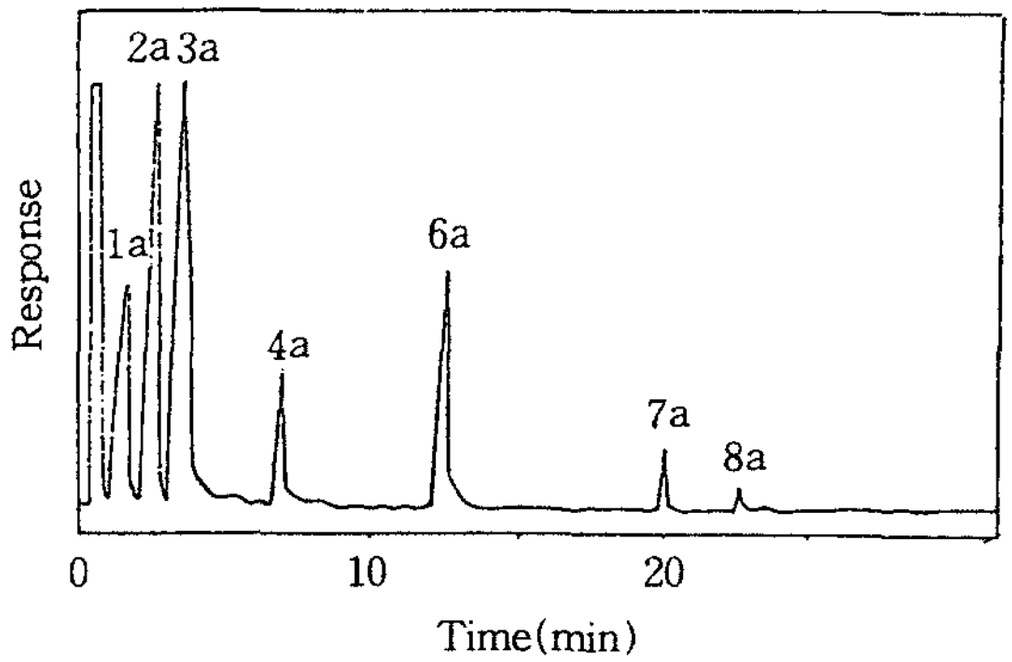

Fig. 2. GC of methyl esters of fatty acids from E. coli LPS.

Peaks designated as la to 4a and 6a to 8a are the corresponding peaks in the Fig. 1.

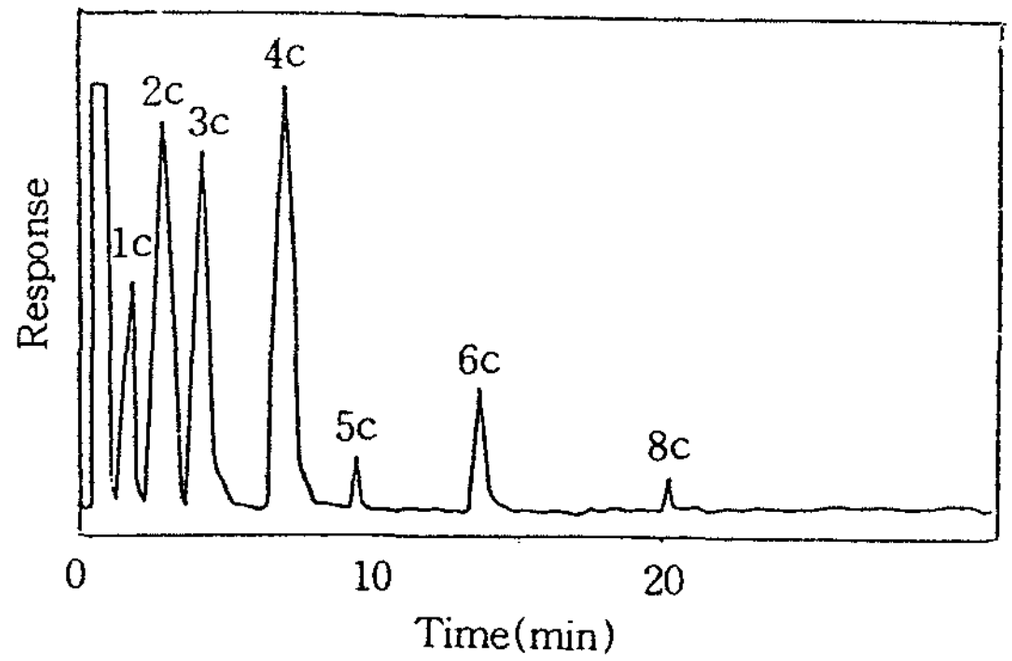

Fig. 4. GC of methyl esters of fatty acids from V. vulnificus LPS.

Peaks designatede as 1c to 6c, and 8c are the corresponding peaks in the Fig. 1.

unique points in the fatty acid compositions of LPSs were that myristic acid was composed as important one(41.37%) for *V. vnlnificus* LPS and that the amount of palmitic acid(C16 : 0) was very small(below 2%) for all LPSs. But *V. vulnificus* LPS was similar in overall fatty acid compositions to those of *E. coli* and *S. typhimurium* LPSs.

It was reported that the low biological activity of *Agrobacterium sp.* LPS might be due to its different chemical composition.[8] In contrast to the typical fatty acid pattern of the enterobacterial LPS(3-hydroxy myristic acid accompanied by lauric, myristic, and palmitic acid), the fatty acids of *Agrobacterium* sp. LPS were consisted solely of the two 3-hydroxy fatty acids(3-hydroxy myristic and palmitic acid) and was devoid of heptose and contained relatively little 2-keto -3-deoxy octonate(KDO). On the other hand the removal of fatty acids esters from lipid A by

Table 1. Fatty acid compositions of *V. vulnificus*, *E. coli*, and *S. typhimurium* LPSs

Fatty acid	% of fatty acids from LPS		
	V. vulnificus	*E. coli*	*S. typhimurium*
$C_{8}:0$	7.62	9.59	6.34
$C_{10}:0$	24.79	31.81	48.60
$C_{12}:0$	19.77	37.03	24.99
$C_{14}:0$	41.37	6.21	2.59
$C_{16}:0$	1.13	–	1.84
$C_{17}:0$	4.38	11.65	9.13
$C_{18}:0$	–	2.92	0.26
$C_{18}:1$	0.94	0.79	–
$C_{18}:2$	–	–	1.06
$C_{18}:3$	–	–	5.19
$C_{20}:0$	–	–	–

– : not detectable

mild alkaline hydrolysis substantially reduced the biological activity of LPS. Therefore it might be said that the biological activity of LPS depended on its fatty acid composition.

The fatty acid compositions of three LPSs were analyzed in order to study the fatty acid characteristics of *V. vulnificus* LPS and compared that of *V. vulnificus* LPS with those of reference *E. coli* and *S. typhimurium* LPSs in relationship of the biological responses. The main fatty acid for each LPS was lauric acid for *E. coli*, capric acid for *S. typhimurium*, and myristic acid for *V. vulnificus* LPS(Table 1). *V. vulnificus* LPS had been expected to have the greatest activity of three LPSs because the chain of myristic acid was the longest.

Ⅳ. Conclusion

1. *V. vulnificus* lipopolysaccharide(LPS) was extracted and analyzed the fatty acid composition. This result was compared to those of *E. coli* and *S. typhimurium* LPSs.

2. The most abundant fatty acid of *V. vulnificus* LPS was myristic acid(C14 : 0), *E. coli* LPS was lauric acid(C12 : 0), and *S. typhimurium* LPS was capric acid(C10 : 0), but above three fatty acids were the major components for all three LPSs (above 70%).

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