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ANALYSIS OF FATTY ACIDS OF MYCOPLASMA CELL

By

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SUMMURY

Gas chromatography was performed for fatty acid analysis of lipid extracts of 9 antigens of *M. bovis, M.bovigenitalium, M. bovirhinis, M. arginini, M. alkalescense, M. canadense, M. capricolum, A. laidlawii* and *Ureaplasma* species. It was found that, there was characteristic differences in number and kind of fatty acids between the examined mycoplasma that could be posibly used as finger print for the identification of mycoplasma species.

INTRODUCTION

All mycoplasma lipids are associated with the cell membrane (Smith, 1979). Mycoplasma is the only prokaryote that requires external sterol source for growth (Razin, 1978) that is why the lipid content of the membrane varies among the various species and depends on the phase of growth and on growth medium (Razin, 1978). Substantial changes in membrane lipid content of many mycoplasma species were observed varying the serum concentration of growth medium (Rottem and Markowitz, 1979), also lipid content was decreased as cells proceed from the early logarithmic to the stationary phase of growth (Rottem and Greenberg, 1975).

The present study is conducted to analyze the fatty acids of different mycoplasma cells to show its possible use as finger print for the identification of mycoplasmas.

MATERIALS AND METHODS

Type cultures:

Seven mycoplasma type cultures, *M.bovis, M. bovigenitalium, M. bovirhinis, M. arginini, M. alkalescense, M. canadense and M. capricolum* and one Acholeplasma laidlawii and one *Ureaplasma* sp. were kindly supplied by Dr. Shin, Diagnostic lab., they were previously isolated from ruminants.

Antigens for these mycoplasma cultures were prepared according to Bois et al. (1984) using mycoplasma medium containing horse serum as described by Hayflick (1965) and ureaplasma medium according to Shepard and Lanceford (1976).

Lipid extraction was performed according to Bligh and Dyer (1959). One volume of cells was added to 2.5 volumes of methanol and 1.25 volumes of chloroform were shaken well for 2 minutes and left at room temperature for 30 minutes, centrifuged at 5000 for 5 minutes and supernatant fluid was separated from the pellet. 1.25 volumes of deionized water were added to the supernatant fluid which contained the lipids; shaken well for 30 seconds and centrifuged. The upper phase was removed and lower phase was evaporated to dryness under a stream of nitrogen. Fatty acid analysis was performed by gas chromatography. Two ml borontrifluoride (14%) in methanol was added to dry lipid samples containing 0.25-0.5 mg lipids. Lipid solution was cooled and 2 ml deionized water was added. The resulting methyl ester was extracted twice each time with 3 ml n-hexane. Methyl ester extractions were combined and hexane was evaporated under nitrogen. Fatty acid methyl ester was redissolved in minimal volume 100 ul of n-hexane and fatty acid methyl ester was analyzed by gas chromatography. Identity of fatty acid methyl esters was done by their retention time relative to that of standard ester mixture.

The gas chromatography used was MIDI-MIS (Microbial Identification, Inc., New York, Delaware - Microbial Identification System).

This study was performed at the Diagnostic Lab., Cornell University, Vet. School, U. S. A.

RESULTS

From the fatty acid analysis of *M. bovirhinis* demonstrated in Table (1), it is clear that *M. bovirhinis* contains many fatty acids, e. g. C14 myristic, palmitic C16, palmitoleic C16:1, stearic C18, C18:2 linoleic, C181 oleic and C20 arachidic and is characterized by containing C18:0, C20:0 arachidic and C20:1 gadoleic acid.

Table (1) showed that *M. bovigenitalium* contains palmitic acid C16, stearic acid C18:0 C18:1 oleic acid and C182 linoleic. It is characterized by having no myristic C14, C17 and palmitoleic C16:1.

It is clear that *M. bovis* contains myristic acid (C14), palmitoleic C161, palmitic C16, stearic C18, oleic C18 and linoleic C182, C17.

It was found that *M. alkalescense* contains myristic acid (C14:0), stearic C18, linoleic C192 and gadoleic C20:1.

A. laidlawii fatty acid analysis showed that it contains myristic acid C14, palmitic acid C16, palmatoleic acid C16:1, oleic acid C18:1 and linoleic acid C18:2.

Table (1) also showed that *M. arginini* contains myristic acid C14:0, linoleic C18:2, stearic C18:0 and gadoleic acid C20:1.

From *M. canadense* fatty acid analysis demonstrated in Table (1), it is found to contain palmitic acid C16, linoleic C18:2, stearic C18 and C19:1.

M. capricolum contains palmitic acid C16, linoleic C18:2, oleic C18:1 and stearic C18.
Table (1) showed that ureaplasma contains linoleic acid, C20 and C9:5.

Table (1): Analysis of fatty acid of mycoplasma cell

Species	Fatty acids	Species	Fatty acids
M. bovirhinis	C14	*M. bovigenitalium*	C16
	C16		C18
	C16:1		C18:1
	C18		C18:2
	C18		
	C19:1		
	C20:0		
	C20:1		
M. bovis	C14	*M. alkalescence*	C14:0
	C16		C18
	C16:1		C18:2
	C18		C20:1
	C18:1		C16:0
	C18:2		C16:1
	C17		C18:1
A. laidlawii	C14	*M. arginini*	C14:0
	C16		C18:1
	C16:1		C18:0
	C18:1		C20:1
	C18:2		
M. canadense	C16	*M. capricolum*	C16
	C18:2		C18:2
	C18		C18:1
	C19:1		C18
Ureaplasma sp.	C18:2		
	C20		
	C9:5		

DISCUSSION

Mycoplasma lipids including fatty acids were studied by few investigators such as (Rottem and Greenberg (1975) for *M. homonis*, Bovers et al (1977) for *A. laidlawii*, Rottem and Markowitz (1979) for *M.gallisepticum*, Smith (1979) for *M. pneumoniae*, Rottem (1980) for *Spiroplasma* and *Ureaplasma*, Thomas and Sharp (1990) referred to the variation of lipid content among different M. gallisepticum strains.

The present study is considered One of the Few approaches to study the lipids of mycoplasma, it is conducted to analyze the extract of 7 Mycoplasmas, 1 Acholeplasma and 1 Ureaplasma. It was found that there is difference in fatty acid composition among them, e. g. M. bovigenitalium is characterized by the absence of myristic, C17 and palmitoleic, *M. bovirhinis* is characterized by having C19, C20 (unsaturated acid arachidic) and gadoleic. All the examined mycoplasma contained linoleic acid, palmitic, oleic and stearic except

ureaplasma. From the results of this study, fatty acid analysis of mycoplasma could be used for the identification regarding that the medium for the homologous strains and time of in cubation are the same.

REFERENCES

Bevers, E. M.; S. A. Singal; J. A. F. Opden Kamp and L. M. Van Deenen. (1977): Recognition of different pools of phosphatidylglycerol in intact cell and isolated membranes of Acholeplasma laidlawii by phospholipase. Az. Biochemistry, 16 No. 7: 1290-1295.

Bligh, E. G. and W. J. Dyer (1959): A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Boss, J. M.; M. Roy and R. S. Guay (1984): Counter immunoelectrophoresis for the diagnosis of bovine mycoplasmosis. Am. J. Vet. Res. 45, 5, 1020-1022.

Hayflick, L. (1965): Tissue culture and Mycoplasma. Tex. Rep. Biol. Med. 23: 285-303.

Razin, S. (1978): The Mycoplasmas. Microbiol. Rev. 42, 2, 414-470.

Rottem, S. and A. S. Greenberg (1975): Changes in composition, biosynthesis and physical state of membrane lipid occurring upon aging of M. hominis culture. J. Bact. 12, 2, 631-639.

Rottem, S. and O. Markowitz (1979): Membrane lipids of M. gallisepticum. A distributed phosphatidylcholine and a phosphatidylglycerol with an unusual positional distribution of fatty acids. Biochemistry, 18 No. 14/2, 930-935.

Rottem, S. (1980): Membrane lipids of spiroplasma and ureaplasma. In Methods in Mycoplasmology, Vol. I (1983), Acad. Press, N. Y.

Shepard, M. C. and C. D. Lanceford (1976): Differential agar medium A7 for identification of Ureaplasma urealyticum in primary culture of clinical material. J. Clin. Microb. 3, 6, 613-625.

Smith, P. F. (1979): The composition of membrane lipids and lipopolysaccharides. In the Mycoplasma, Vol. I M. F. Barile and S. Razin, Acad. Press, pp. 231-257.

Thomas, C. B. and P. Sharp (1990): Glycoconjugate heterogeneity among five strains of Mycoplasma gallisepticum. Av. Dis. 34, 969-978.