Arch. Inst. Razi, 1967, 19, 87 - 90

MYCOPLASMA AGALACTIAE

I–CHEMICAL COMPOSITION OF *M. AGALACTIAE* (*)

by

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Mycoplasma agalactiae is the causative agent of contagious agalactia in sheep and goats. The economic loss caused by this disease in Iran is very important. For this reason we were interested to discover the chemical composition of M. agalactiae for our future studies.

MATERIALS AND METHODS

Mycoplasma agalactiae strain: The M. agalactiae strain used for this study isolated from a milk sample of a mastitis affected sheep. It was preserved in serum broth.

Cultivation Medium: To prepare a crude starting material the M. agalactiae was cultivated in Difco PPLO Broth w/o CV medium. This broth was supplemented with 20% of horse serum, 1% of yeast extract (BBL) and 500 units of Penicillin per ml of medium. The culture was incubated for 36 hours on a shaker apparatus in the 37° C incubator. The organisms were then sedimented from the medium by centrifugation in the Sorvall Continuous-Flow centrifuge at 15,000 rpm.

Analysis: The sediment was washed several times in 0.15 M Phosphate buffered saline (PBS), pH=7.2. The washed sediment was diluted in demineralized water and disintegrated by X-Press apparatus (5) and lyophilized by a Stokes Freeze-Drying Machine. The lyophilized material was weighed and extracted by various chemical procedures in order to determine the chemical composition of the Microorganism. The cellular nitrogen was estimated by the Micro-Kjeldahl method de-

* Supported in part by NIH Research Grant No. 1 RO5 TWOO238 — 01.

scribed by Morris B. Jacob (8). The protein was precipitated by 5% Trichloracetic acid (TCA).

The lipid fraction of the supernatant was extracted with ethanol-ether (3:1) by using the Bloor (1) and Boyd (2-3) methods, evaporated to dryness, then dissolved in Chloroform. The lipid fraction of the residue was extracted with acetone, evaporated to dryness, then extracted with anhydrous ether. Cholesterol was determined by the Liebermann-Burchard reaction and the Cholesterol esters were estimated by a modification of the technique by V. Harlay (7). Phosphorus was estimated by the method of Fiske and Subbarow (6). Extraction of nucleic acids was carried out with hot TCA as described by Schneider (9). The methods of Burton (4) and Schneider (9) were used for estimation of DNA and RNA respectively.

RESULTS

A summary of the data obtained to date is shown in Table I. Each of these values is based on several different determinations. In this table it can be seen that the protein makes up more than 50% of the dry weight of the organism. An interesting finding in this table is the presence of Sterol in the lipid fraction. The other interesting feature of this fractionation is the amount of nucleic acids that are estimated to comprise nearly 7 per cent of the dry weight of this organism. Analysis of these nucleic acids show that pentose is present in an amount twice that of the desoxypentose.

Table I — Chemical analysis of Mycoplasma agalactiae

Fractions	Mg/100mg dry weight
Total Cellular Nitrogen	11.2
Protein (N.P. X 6.25)	59.8
Nucleic Acids :	6.63
Pentose	4.60
Desoxypentose	2.03
Lipid :	5.10
Phospholipid	0.47
Sterol	2.0
Fatty Acids	2.35

Table II shows the result of analysis of the lipid fraction of this microorganism. From this table it appears that the amount of free sterol in the residue is higher than that present in the supernatant fraction. It can also be noted that free sterol is approximately twice as prominent as the bound sterol in this microorganism. As regards the phosphorus status, the supernatant contains more phospholipid, whereas the level of acid-soluble phosphorus is higher in the residue.

Table II — Analysis of lipid fraction of M. agalactiae

Cell Fraction	Sterol Mg/Gm dry weight		
	Free	Bound	Total
Whole	24.55	11.70	36.25
Residue	18.25	10.50	28.75
Supernatant	6.30	1.20	7.50
	Phosphorus-containing components Mg/Gm dry weight		
	Phospholipid	Acid-soluble phosphorus	
Whole	9.54	13.20	
Residue	5.04	12.08	
Supernatant	4.50	1.12	

DISCUSSION

In this study, a number of chemical fractions were separated from disintegrated suspension of Mycoplasma agalactiae. Since the organism was grown in a broth medium containing beef heart infusion, yeast extract and horse serum, the possibility of various fractions being contaminated with growth medium constituent cannot be excluded. To minimize such contamination, the deposited organisms were washed several times, but, some degree of contamination may still occured.

SUMMARY

The chemical composition of Mycoplasma agalactiae has been determined. The composition of nucleic acids of this organism and also the amount of lipid (5%) in the dry weight of the cells is similar to that of other bacteria. However, a distinguishing feature of this organism is the presence of sterol similar to cholesterol and cholesterol ester in its lipid fraction.

REFERENCES

1— Bloor, W.R. (1928)
The determination of small amounts of lipid in blood plasma.
J. Biol. Chem., **77**, 53

2— Boyd, E.M. (1936)
The extraction of blood lipid.
J. Biol. Chem., **114**, 223

3— Boyd, E.M. (1936)
The extraction of lipid from red blood cells.
J. Biol. Chem., **115**, 37

4— Burton, K. (1956)
A study of condition and mechanism of the Diphenylamine reaction for colorimetric estimation of Desoxyribonucleic Acid (DNA).
Biochem. J., **62**, 315-323
5— Edbo, L. (1960)
A new press for the disruption of Micro-organisms and other cells.
J. Biol. Micro. Techn. Eng., II, No. **4**, 453-479
6— Fisk, C.H. and Subbarow, Yellapragada (1925)
The colorimetric determination of Phosphorus.
J. Biol. Chem., **66**, 375
7— Harlay, V.
Fiches techniques de chimie biologique du Prof. Fleury.
Les Edition Véga 175, Boulvard St. German, Paris 6ème.
8— Jacob, M.B. (1951)
Micro-Kjeldahl for biologicals.
J. Am. Phar. Asso., XI, No. **3**, 151-153
9— Schneider, W.C. (1945)
Phosphorus compound in Animal tissues.
I— Extraction and estimation of Desoxypentose nucleic and Pentose nucleic acid.
J. Biol. Chem.. **161**, 293-303.