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UP TAKE OF GLYCOLIPIDS BY MYCOPLASMA CAPRICOLUM MEBRANES

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ABSTRACT: Mycoplasma capricolum was grown with and without DGDG containing cholesterol on Edwards modified media. Total lipids were extracted with chloroform: methanol (2:1). Membrane protein and phospholipids were determined by Lowery and Chen methods respectively. Total lipids were separated on TLC and glycolipids were located with iodine while diphenylamine and orcinol test was performed for lipid sugar. Three positive sugar spots for microorganisms grown on DGDG and cholesterol were found. Either DGDG is being metabolized to new components or new glycolipids are being synthesized from DGDG.

Key words: Glycolipids, Mycoplasma, Capricolum, Membranes.

INTRODUCTION: **G**lycolipids of Myco-plasma pneumoniae lipid extract have been identified [1]. Characterization of membrane lipids of Mycoplasma mobile has been undertaken [2]. Sulphated glycolipids of Mycoplasma hominis a human pathogen have been isolated [3]. Chemical composition and trans bilayer distribution of Mycoplasma membrane lipids has been studied [4]. Physical studies of lipid orgnization and dynamics in Mycoplasma membrane have been described [5]. Choline containing glycolipids in Mycoplasma fragmemnt PG-18 has also been found [6]. Existence of glycolipids micro domains with both medel and cellular membranes has been documented [7]. Mf-GL-II has been considered as major lipid membrane of AID associated Mycoplasma [8]. Glycolipids of similar mobility of themus strains have been characterized from Meiothermus spp [9]. Complement-C activation by various bacterial surface glycolipids has been reported [10].

MATERIAL AND METHODS:

Growth of Acholeplasma laidlawii- B. Acholeplasma laidlawaii-B was grown statistically in lipid depleted bovine serum albumin meidum. The medium was supp-lemented with 0.06M oleic acid plus 0.06M plamitic acid added as sterile ethnolic solutions. Growth was followed by absorance at 640nm. The cells were harvested by centrifugation after 20 hours at 37°C. Membranes were prepared by osmotic lysis and washed [11].

Growth of Mycoplasma capricolum: Mycoplasma capricolum was grown under identical conditions with and without the supplementation of DGDG in Edward's modified media. It was grown on medium containing cholesterol 10µg/ml and DGDG 10µg/ml with cholesterol 2µg/ml. Mycoplasma Capricolum (California Kid strain 14, ATCC 27342) was cultured on modified Edwards medium [11]. After tha bacto-heart infusion broth, bacto-peptone and yeast extract component has been exhaustively extracted with chloroform: methanol (2:1). Thallium acetate was omitted and PPLO serum fraction was replaced with 4mg/ml essentially fatty acid free bovine serum albumin (Fraction V, Sigma) [12]. Mycoplasma capricolum was grown under identical conditions in medium on cholesterol 10µg/ml and of DGDG 10µg/ml with cholesterol µg/ml separately. Ethanolic solutions of cholesterol 10µg/ml and fatty acids, palmitic 5µg/ml and oleic 6.4µg/ml was mixed and added to the medium. Microorganisms were grown statically at 37°C for 24 hours after which the cells were harvested and total lipids were obtained.

Extraction of Total Lipids:

Total lipids were extracted thrice with chloroform: methanol (2:1) [13]. Non-lipid components were removed by washing over a Sephadex G25 column [14]. Membrane protein and phospholipids were determined by Lowery [15]. And Chen et. al method [16] respectively.

Characterization of Lipids: Total lipid extracted was separated on silica gel H plates using chloroform: methanol: water, 65:24:4 (V/V/V) [17]. And MGDG, DGDG and PG as markers. Glycolipids were located with iodine vapours and lipid sugars identified by diphenylamine reagent and orcinol test [18], [19]. MGDG and DGDG were scraped and eluted with chloroform: methanol (2:1) and their sugars contents were determined [20].

RESULTS AND DISCUSSION

When Mycoplasma capricolum was grown under identical conditions with cholesterol (10µg/ml) or with DGDG (10µg/ml) plus cholesterol than glycolipid, DGDG. However, addition of small amounts of cholesterol (2µg/ml) to DGDG (10µg/ml) had positive effect on the growth of Mycoplasma capricolum (Figure 1 and 2). A decrease in protein and phospholipids without effecting glycolipid contents of cells was observed when the organisms were grown on DGDG 10µg/ml with cholesterol 2µg/ml (Table-2). Similarly membranes prepared after lysis of the cells grown on DGDG with cholesterol also showed a decrease in protein and phospholipids contents but no effect on glycolipids was recorded (Table-2. Total lipids extracted from the cells raised on DGDG (10/ml) containing cholesterol (2/ml) also showed similar results where a decrease in protein and phospholipids and no effect in glycolipids amount was seen (Table-2). It is evident from Table-2 that the concentration of glycolipids of Mycoplasma capricolum raised either on cholesterol or on DGDG with cholesterol persisted in cells, membranes and total lipids.

Separation of total lipids on TLC plates using markers MGDG, DGDG and (Phosphoglycerol) and development after

spraying with orcinol and diphenylamine reagent showed the presense of a number of bands. Two bands were located when Mycoplasma capricolum was on cholesterol (10µg/ml) and four bands were observed when DGDG (10µg/ml) were supplemented with cholesterol (2µg/ml). On cholesterol grown organisms, one of the two bands one correspondent to the marker PG while the identity of other which was positive for sugar remains to be determined. Among the four bands when organisms raised on DGDG with cholesterol, one was tentatively identified as MGDG.

Glycolipids containing both phosphorus and carbohydrate residues have been isolated from a wide variety of bacteria [21-28]. Prior to these reports representative of this type of lipids were restricted to the family of phosphatidyl inositol mannosides 29 and glucosaminyl phosphatidyl glycerol present in Bacillus magnetarium and pseudomonas ovalis [30-32]. An analogues structure, diglucosyl phosphatidyl glycerol has been proposed for the glcophospholipids subsequently isolated from Mycoplasma laidlawii and originally called phosphatidyl glucose [33]. Has been revised to glycerol phosphoryl diglyceride based upon DGDG [23].

The origination of new components giving positive orcinol test in the lipids of Mycoplasma capricolum grown on DGDG (10µg/ml) with cholesterol (2µg/ml) hence can virtually be interpreted well. According to Rigau and Leblance [34]. The adaptation with little cholesterol increases the amount of diacyl glycerides in their membrane. Moreover we are making use of DGDG but there are no glycolipids in Mycoplasma capricolum therefore either DGDG is being metabolized in to new components or new glycolipids are being synthesized from DGDG. The actual fate of DGDG is unclear at this stage.

Table-1: Glycolipids Obtained From Acholeplasma Laidlawii B and Their Sugar Contents.

Glycolipid	Milligrams	Millimoles (mM)Sugar(mg	Sugar(mM)
MGDG 14.7	0.019+	7.75	0.043*
DGDG 6.3	0.007+	1.56	0.009*

+ On the Basis of MGDG or DGDG *On the basis of Glucose

Table-2: Proteins, Phospholipids and Glycolipids Contents of Mycoplasma Capricolum Given in Milligrammes.

	Cells	
	Grown on Cholesterol	**Grown on DGDG with Colesterol***
Protein	1.83**	0.46**
Phospholipids	1.18	0.40
Glycolipids	0.26	0.28
	Membranes	
Protein	1.11	0.35
Phospholipids	0.94	0.34
Glycolipids	0.26	0.28
	Total Lipids	
Protein	0.33	0.04
Phospholipids	0.25	0.19
Glycolipids	0.26	0.28

* DGDG 10µg/ml and Cholesterol 2µg/ml **mg/mg of Protein

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