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Respiration Activity and Carbohydrate-Lipide Content during Growth and Starvation of *Mycobacterium* sp. 279

Aktywność oddechowa oraz zawartość węglowodanów i lipidów podczas wzrostu i głodzenia *Mycobacterium* sp. 279

Респираторная активность, содержание углеводов и липидов во время роста и голодания *Mycobacterium* sp. 279

A genetic peculiarity of mycobacteria is a high proportion of storage materials, chiefly lipids and carbohydrates, that are located mainly in a very thick and hydrophobic cell wall structure (1). The level of the storage materials depends on the age of the mycobacterial culture, the composition of the culture media and other factors (ref. Tepper, 1965). As a consequence, an enormously high and variable endogenous respiration of the cell suspension is observed which causes difficulties in the proper interpretation of the substrate utilization by the particular cultures when manometrically tested. By using various kinds of starvation techniques the central well contained 0.2 ml of 20% KOH. Equilibration lasted 10 min. and reading was talsen every 10 min. during 90 min. of the incubation.

This report compares the changes in lipide and carbohydrate content with the respiration activity during the growth and starvation of *Mycobacterium* sp. 279.

MATERIALS AND METHODS

The organism employed was a *Mycobacterium* sp. 279. The strain was obtained from the Department of Plant Physiology, University in Lublin and was maintained in this Laboratory on appriopriate media by serial mass transfers. It was grown on the surface of a glucose-glutamate-citrate-salts medium (2) at 37°C for several days. After incubation, cells were harvested and washed with water by centrifugation.

Starvation was carried out with an underlayering technique (3) by incubating washed cultures for definite times in the growth medium with glucose omitted.

Oxygen uptake was measured manometrically at 37°C with the conventional Warburg method. Each Warburg vessel contained in the final volume of 2.5 ml; 1 5 ml of 0.1 M phosphate buffer (*pH* 7.0), 1 ml of the cell suspension (6 mg dry weight) and 0.5 ml of 0.1 M substrate solution (or 0.5 ml of water in controls). The central well contained 0.2 ml of 20% KOH. Equilibration lasted 10 min and reading was talsen every 10 min during 90 min of the incubation.

RESULTS AND DISCUSSION

The manometric method is frequently employed to screen the substrate utilization by microorganisms in metabolic and taxonomic studies. This technique was also successfully applied to follow the enzyme induction and to distinguish inductive enzymes from constitutive ones (3).

Mycobacteria are rather inconvenient for the manometric determinations due to a high level of their stored materials which undergoes fluctuation depending upon growing conditions. A characteristic feature is their substantial endogenous respiration, hence low or negligible response to various compounds. In many cases it looks like a particular compound is not a substrate for mycobacterial cells, despite its evident uptake from the incubation mixture.

S z y m o n a and S z y m o n a (3) were the first routinely to apply different starvation procedures to greatly reduce the stored materials and simultaneously to diminish their endogenous respiration. We have observed (unpublished data) that the lowering of a carbon substrate concentration (mainly glucose and glycerol) in the growth medium of *Mycobacterium* sp. 279 (i.e. from 4 to 2%) resulted in the obtention of less slimy cultures with the reduced endogenous metabolism, especially when they come up to an early stationary phase of growth.

Table 1 shows that 3-day old cultures of *Mycobacterium* sp. 279 which are thought to be in the exponential phase of growth reveal an elevated endogenous metabolism and low response to added glucose and lactate. The prolongation of cultivation to 4 days, presumably to reach the early stationary phase of growth, results in the decrease of endogenous respiration by approx. 35% with simultaneous 2-fold and 3-fold elevation of the response to glucose and lactate, respectively. The extention of incubation to 5 days causes further drop in the endogenous respiration with the response to the added substrates unchanged or slightly diminished. However, between the fifth and seventh day of cultivation a considerable drop in the respiration activity of cells is observed.

The changes in the carbohydrate content of the cultures roughly parallel the variations in the endogenous respiration. Thus, 6-day old cultures possess 43% less carbohydrates than 3-day old ones. The reason was

Table 1. Effect of growth age on total carbohydrate content and respiration activity of *Mycobacterium sp.* 279

Growth age /days/	Glucose conc. in medium /%/	Total carbohydrate content /mg/g dry wgt/	Respiration /$\mu l O_2$/h/mg dry wgt/				
			Endogenous	D-Glucose	D-Glucose /"extra"/*	DL-lactate	DL-lactate /"extra"/*
0	2.00						
3	1.10	142	44.8	78.4	33.6	63.6	18.8
4	0.45	108	29.0	95.0	66.0	87.3	58.3
5	0.15	95	14.0	62.0	48.0	72.0	58.0
6	0.03	82					
7	0.00	82	5.2	16.1	10.9	11.2	6.0

* After subtraction of the endogenous respiration.

virtually in the exhaustion of glucose from the media. In fact, 5-day old cultures were totally devoid of the mentioned source of carbon and energy and feel into starvation. The accelerated drop in the bacterial respiration at the end of cultivation may be explained as a result of an impairment of metabolic activities brought about by the alkalinization of the media. Namely, it was checked that the pH-value of the media rose from 7.8 for 5-day old cultures to 8.5 for 7-day old cultures, as a result of the intensive deamination of glutamate, a possible substitute for exhausted glucose.

Table 2 shows determinations of the respiration activity and the lipide — carbohydrate content of *Mycobacterium* sp. 279 from 4% glucose during staravtion on a sugar-free medium. As can be seen, 24-hour culture starvation was manifested with the diminution of the endogenous meta-

Table 2. Effect of starvation on carbohydrate-lipide content and respiration activity of *Mycobacterium sp.* 279

Analysis	Starvation time /hours/					
	0	1.5	3	6	12	24
Endogenous respiration*	36.8	24.1	18.8	17.2	11.8	8.4
Respiration with glucose*	84.5	73.5	69.3	70.5	68.0	64.5
"Extra" respiration* **	47.7	49.4	51.5	53.5	56.2	56.1
Total carbohydrates***	166.0	150.0	134.0	124.0	110.0	88.0
Total free lipids***	353.0			324.0	267.0	242.0

4-Day old cultures from 4% glucose medium were used. Starvation was performed with the underlayering technique on sugar-free growth medium.

* Respiration expresed in µl O_2/h/mg dry wgt.

** Respiration after subtraction of the endogenous respiration.

*** Carbohydrate and lipide content of cells expressed in mg/g dry wgt.

bolism by 80% and the augmentation of the response to added glucose. During that period carbohydrates were burnt by one — half and lipids by 30%.

It may be stated in conclusion that the starved cultures of *Mycobacterium* sp. 279 or the cultures from scanty synthetic media and harvested in the early stationary phase of growth appear to be suitable material for the manometric determinations since their reduced endogenous metabolism which reflects the diminished pool of the stored materials, chiefly carbohydrates and lipids.

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STRESZCZENIE

Niepatogenny szczep *Mycobacterium* sp. 279 hodowano na syntetycznym podłożu zawierającym 2% glukozę jako źródło węgla i energii, a następnie analizowano komórki na zawartość całkowitych węglowodanów i aktywność oddechową, zaś podłoże — na zawartość glukozy. Stwierdzono, że w miarę wyczerpywania się glukozy z podłoża występuje spadek węglowodanów oraz obniżenie się metabolizmu endogennego komórek. Równocześnie rośnie reakcja komórek w aparacie Warburga na dodane z zewnątrz związki, które komórki potrafią metabolizować. Głodzenie komórek na podłożu bezcukrowym również powoduje obniżenie oddychania endogennego w rezultacie wyczerpywania się substancji zapasowych w formie węglowodanów i lipidów, dzięki czemu reakcja na dodane z zewnątrz związki jest wyraźna. Tak więc mykobakterie z podłoża syntetycznego, zawierającego obniżone stężenie glukozy, i zebrane we wczesnej fazie stacjonarnej oraz mykobakterie głodzone na podłożu bezcukrowym są dobrym materiałem do oznaczeń manometrycznych.

РЕЗЮМЕ

Исследовано штамм *Mycobacterium* sp. 279 выращиваемый на стандартной питательной среде. При помощи манометрических определений показано, что высокий эндогенный метаболизм является результатом выступления большого количества запасных веществ, главным образом липидов и углеводов. Определено низкую реакцию штамма на добавление субстратов. Предыдущее голодание на безсахарной среде ведет к значительному снижению запасных веществ, снижению респираторной активности и повышению реакции на субстраты.