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A new defined medium for cultivating *Leishmania* promastigotes*

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Summary

A chemically defined medium to grow the promastigotes of 19 stocks of *Leishmania* is described. The medium was developed by making qualitative and quantitative modifications of the medium AR-103 devised for *Trypanosoma cruzi*. The new medium, designated as MD-29, can be used in studies on the nutritional requirements of promastigotes.

Key words: *Leishmania*; cultivation of *Leishmania* promastigotes; chemically defined culture medium.

Introduction

The complexity of the media used to cultivate members of the genus *Leishmania* has complicated the feasibility of comparative studies on nutritional requirements, metabolism and other characteristics of these organisms, especially those isolated from mammals. The cultivation of *Leishmania* in a defined medium opens up new possibilities to determine biochemical and immunological characteristics.

A chemically defined medium (AR-130) for the cultivation of *Trypanosoma cruzi* was developed (Azevedo and Roitman, 1983); quantitative and qualitative (ATP and AMP were omitted and iron nitrate was added) modification of this medium led to the design of a chemically defined medium, herein designated as MD-29 in which several strains of *Leishmania* can be cultivated.

* This work is part of a Thesis for the Doctor Degree in Parasitology, by the senior author.

Table 1. Defined medium MD-29 for *Leishmania* cultivation

Components	mg/l	Components	mg/l
Fructose	2000	D Choline	12
A $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$	5000	E L-Alanine	285
β -glycerophosphate Na salt ..	20000	L-Arginine	550
Sodium acetate $3\text{H}_2\text{O}$	540	L-Aspartic acid	100
NaCl	4000	L-Cystein HCl	100
KCl	400	L-Glutamic acid	1045
EDTA Na salt	80	L-Glycine	350
Trisodium citrate $2\text{H}_2\text{O}$	600	L-Histidine	350
$\text{FeNO}_3 \cdot 9\text{H}_2\text{O}$	0.0000005	L-Isoleucine	260
Sodium succinate $6\text{H}_2\text{O}$	270	L-Leucine	760
B Adenosine	20	L-Lysine HCl	750
Adenine HCl	50	L-Methionine	175
Guanosine	20	L-Phenylalanine	373
Guanine HCl	1.5	L-Proline	780
Hypoxanthine	1.5	L-Hydroxyproline	50
Xantine	1.5	L-Serine	285
C Folic acid	30	L-Threonine	270
D- α tocopherol succinate ...	4	L-Tryptophan	140
DL- α lipoic acid	0.4	L-Tyrosine etil ester	360
Nicotinic acid	0.125	L-Valine	485
Nicotinamide	10	F Haemin	1.0
D-Biotin	10	Double distilled H_2O to	
Riboflavin	1	make up to	1000 ml
Inositol	2	pH adjusted to 7.5 with 1 <i>n</i> HCl	
D-Ca pantothenate	10		
Pyridoxine HCl	0.125		
Pyridoxal	10		
Thiamine	10		
Ascorbic acid	0.25		

Notes: All ingredients were obtained from Sigma Chemical Company A. B. C. and E were added from a dry mix stored at -4°C . D was added from a frozen stock solution. The medium was Seitz filtered before an autoclaved solution of haemin (F) was added aseptically. Fructose was added to the completely prepared medium.

Material and Methods

The composition of medium MD-29 is given in Table 1. Nineteen strains of *Leishmania* have been cultivated in medium MD-29. Cultures in the chemically defined medium were initiated with amastigotes from cutaneous lesions (BH6, BH121, BH32, BH132, BH216, BH224, LV78, PH8) and the spleen of experimentally infected hamster (BH46), and from promastigotes grown at 23°C in LIT (liver infusion-tryptose) medium (Camargo, 1964) (BH46, BH121, BH32, BH132, BH216, BH224, LV78, PH8, BH49, LV9, LV4, LV414, LRC-L7, LRC-L121, LRC-L134, LRC-L259, LUMP1482 and LUMP1701).

A fine pointed Pasteur pipette was used to aspirate material from infected tissue. In order to isolate parasites from cutaneous lesions, the animal was anesthetized with chloroform, hair on the

Table 2. *Leishmania* strains cultivated in defined medium MD-29

Designation and source	Species	Place of isolation	Number of passages	Mean growth* ($\times 10^7$ /ml)
BH6 (Man)	<i>L. mexicana mexicana</i>	Brazil	187	4.0
BH46 (Man)	<i>L. donovani</i>	Brazil	208	4.5
BH49 (Man)	<i>L. mexicana</i>	Brazil	152	3.2
BH121 (Man)	<i>L. mexicana</i>	Brazil	218	4.3
LV9 (Man)	<i>L. donovani</i>	Ethiopia	106	3.6
LV4 (<i>Nyctomys sumichrasti</i>)	<i>L. mexicana mexicana</i>	Belize	106	6.0
LV78 (<i>Proechimys</i> sp.)	<i>L. mexicana amazonensis</i>	Brazil	106	4.0
LV414 (<i>Tarentolae</i> sp.)	<i>L. tarentolae</i>	Africa	106	5.3
PH8 (<i>Lutzomyia flaviscutellata</i>)	<i>L. mexicana amazonensis</i>	Brazil	126	3.5
BH32 (<i>Akodon cursor</i>)	<i>Leishmania</i> sp.	Brazil	59	6.1
BH132 (<i>Proechimys dimidiatus</i>)	<i>Leishmania</i> sp.	Brazil	55	3.7
BH216 (<i>Akodon cursor</i>)	<i>Leishmania</i> sp.	Brazil	55	3.3
BH224 (<i>Proechimys dimidiatus</i>)	<i>Leishmania</i> sp.	Brazil	55	3.0
LRC-L7 (Man)	<i>L. tropica</i>	India	50	3.2
LRC-L121 (<i>Agama stellio</i>)	<i>L. agamiae</i>	Israel	52	3.5
LRC-L134 (Man)	<i>L. aethiopica</i>	Ethiopia	50	3.1
LRC-L259 (Man)	<i>L. mexicana amazonensis</i>	Brazil	53	3.4
LUMP1482 (<i>Coendu rothschildi</i>)	<i>L. hertigi hertigi</i>	Panama	51	3.1
LUMP1701 (<i>Coendu</i> sp.)	<i>L. hertigi deanei</i>	Brazil	51	3.2

* Final cell density after 9 days incubation at 25° C

BH = Belo Horizonte

LRC = Leishmaniasis Research Centre – Jerusalem

LV = Liverpool

LUMP = London University Medical Protozoology

PH8 = Obtained from Ralph Lainson – Instituto Evandro Chagas – Belém

border of the lesion was removed, and the skin surface was sterilized with merthiolate before inserting the pipette into the margin of the lesion. When parasites were to be isolated from the spleen, the animal was killed and the abdomen opened to expose the organ. When promastigotes were used to initiate cultures, 1 ml was withdrawn from 48-h LIT cultures containing $2\text{--}3 \times 10^7$ flagellates/ml, and added to the defined medium in 20×150 mm screwcap test tubes to give a final volume of 5 ml.

Subcultures were made at 9-day intervals by transferring 1 ml of the culture to 5 ml of the chemically defined medium. Cultures were incubated at 25° C and growth was assessed qualitatively and quantitatively by observations on the appearance and mobility of promastigotes and by assessing their numbers by means of a Coulter Counter, model ZB.

Results

A summary of the results obtained with 19 strains of *Leishmania* is given in Table 2. The defined medium supports the growth of strains isolated from both vertebrate and invertebrate host, with a yield of more than 3×10^7 promastigotes/ml in a 9-day period.

Discussion

Medium MD-29 can be used for mass produce promastigotes for analytical biochemical studies. In fact, the medium was used already for the production of promastigotes for studies on surface polysaccharides (Fiorini et al., 1981), nutritional requirements (Melo et al., 1982) and other important aspects of in vitro development as the maintenance of the pathogenicity of *Leishmania* (Melo, 1983).

As far as we know, this is the first time that strains of *L. tropica*, *L. aethiopica*, *L. hertigi*, *L. mexicana* and New World *L. donovani* have been cultivated in a defined medium. The defined media previously described (Trager, 1957; Steiger and Steiger, 1976, 1977; Berens and Marr, 1978) were used to culture only *L. tarentolae*, Old World *L. donovani* and *L. braziliensis*. Medium RE III of Steiger and Steiger (1977) did not support the development of *L. tropica*, *L. mexicana texana* or *L. hertigi* (Fish et al., 1978).

Leishmania species seem to be fastidious in their nutritional requirements since many strains of *Leishmania* are not able to grow in a defined medium – containing 10 amino acids, 7 vitamins, purine and hemin – (Roitman et al., 1972), that supports the growth of many lower trypanosomatids (unpublished results).

The availability of a defined medium contributes to an improved understanding of the biology of *Leishmania*. It helps to develop rational methods of chemotherapy; provides a source of cells free of extraneous antigenic material; and may lead to a better knowledge of the genetics of the parasites. The knowledge of the nutritional requirements by using the present medium will allow to design a minimal medium for *Leishmania*. Studies on antigens for immunodiagnosis derived from promastigotes, cultured in our defined medium, are in progress.

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