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COMPARISON OF *MYCOBACTERIUM AFRICANUM*, *M. TUBERCULOSIS* AND *M. BOVIS* BY THEIR UTILIZATION OF CARBON AND NITROGEN SOURCES

by

G. VAN DER GROEN & S. R. PATTYN

Summary — In a minimal medium with glucose as sole carbon source, *M. africanum* could not utilize L-α alanine as sole nitrogen source. In this respect *M. africanum* behaves like *M. bovis* and is different from *M. tuberculosis*.

KEYWORDS : Mycobacterium africanum; Mycobacterium tuberculosis; Mycobacterium bovis; Bacteriological Techniques.

Mycobacterium africanum (Castets *et al.*, 1968) was discovered in our laboratory (Hermans-Boveroulle *et al.*, 1965) and considered « intermediate » between *M. bovis* and *M. tuberculosis*. In a later study, African strains were compared with *M. tuberculosis*, *M. bovis* and *M. microti* (Pattyn *et al.*, 1970) on the basis of cultural and biochemical characters.

In the present study a comparison was made of the different substances *M. tuberculosis*, *M. africanum* and *M. bovis* can use as sole sources of carbon and nitrogen.

Materials and Methods

The strains of *M. tuberculosis* were isolated in our laboratory from local Belgian tuberculosis patients. *M. bovis* strains were local isolates from man or animals. The *M. africanum* strains were dysgonic strains obtained from different parts of Africa and partly included in our previous study (Pattyn *et al.*, 1970).

The bacilli were precultured from Loewenstein-Jensen medium in TB broth (Difco) with 0.5 per cent bovine albumin. They were then inoculated into the basal medium described by Marshak (1951) with the following composition per L : $Na_2HPO_4.12$ Aq. 6.5 g, KH_2PO_4 1 g, $MgSO_4.7$ Aq. 0.02 g, $CaCl_2.2$ H_2O 0.0005 g, $FeCl_3$. 6 Aq. 0.0005 g, Tween 80 0.02 per cent. After sterilisation by heat, filter sterilized bovine albumin is added to a final

concentration of 0.01 per cent. Carbon sources were glycerol, glucose and pyruvate in concentrations indicated in table 1, in the presence of NH_4Cl (1 g/L) as nitrogen source.

Nitrogen sources were NH_4Cl, glutamic acid (Glu), L-asparagine (Asn), L-aspartic acid (Asp), L-alanine (Ala) and L-β-alanine (β-ala) in the presence of 0.1 per cent glycerol as carbon source. The amino-acids were tested in a concentration of 5 μmole/ml.

For each concentration of test substances three tubes with loose metal caps were inoculated and incubated in a water bath at 37 °C. Every second day the tubes were shaken on a vortex mixer for 20″ and measured with a Bausch and Lomb Spectronic 20 photometer at 650 nm. Controls were tubes containing Marshak medium without a carbon or nitrogen source. Results are expressed as the percentage of growth measured in the presence of the C and N sources as compared with the controls devoid of C or N sources.

TABLE 1

Effect of different C-sources on growth of *M. africanum*, *M. tuberculosis* and *M. bovis*

Carbon-source	Initial concn.	% growth (*) *M. africanum*								*M. tuberculosis*	*M. bovis*
	W/V %	2046	3603	3605	3604	3608	3606	3607	Mean	3463	1996
D-Glycerol	0.1	98	103	93	112	92	90	92	97	335	82
	0.25	94	103	80	86	98	87	92	91	310	81
	0.50	87	100	91	92	86	86	93	91	230	90
	1	83	93	85	106	86	84	83	88	230	82
D-Glucose	0.1	107	114	133	109	144	88	30	103	103	140
	0.5	107	60	174	20	60	15	26	66	140	20
	1	124	5	—	35	12	15	28	36	70	9
Pyruvate	0.1	26	126	45	—	130	100	88	86	175	112
	0.5	—	106	4	96	84	12	60	60	161	44
	1	6	48	5	23	50	12	—	24	64	37

$$(*)\ \%\ \text{growth} = \frac{OD\ {}^{650\ nm}_{11\ days} \times 100}{OD\ {}^{650\ nm}_{11\ days\ control}}.$$

Results

Carbon sources

With the exception of glucose and pyruvate at 1 per cent (table 1) the carbon sources in the different concentrations stimulated the growth of *M. tuberculosis.* The growth of the African strains was not stimulated by

the C-sources tested. *M. bovis* is stimulated by D-glucose and sodium pyruvate in a 0.1 per cent concentration.

Nitrogen sources

Table 2 shows that growth of *M. tuberculosis* is stimulated in decreasing order by L-asparagine, L-aspartic acid, glutamic acid, L-alanine and L-β-alanine, the latter two being comparable.

TABLE 2

Effect of different N-sources on growth of *M. africanum, M. tuberculosis, M. bovis*

Nitrogen source	Initial concn. mole/ μml	% growth *M. africanum*								*M. tuberculosis*	*M. bovis*
		2046	3603	3605	3604	3608	3606	3607	Mean	3463	1996
NH_4Cl	5	91	98	60	78	42	—	54	71	177	91
Asn	5	167	132	194	186	179	52	229	163	397	124
Glu	5	208	192	415	363	179	120	193	239	200	119
Glu + Asn	5	159	221	155	216	138	143	177	173	161	217
Asp	5	124	94	230	250	190	—	188	179	370	182
Asp + Asn	5	143	95	93	197	134	111	147	131	125	170
Ala	5	46	30	55	50	39	50	50	46	189	93
Ala + Asn	5	156	151	158	192	157	113	252	168	145	139
β-Ala	5	120	168	138	126	110	90	120	125	166	124

The growth stimulating effect of the amino-acids for *M. africanum* in decreasing order is L-glutamic acid, L-asparagine, L-aspartic acid, L-β-alanine and L-alanine; and for *M. bovis* L-aspartic acid, L-asparagine, L-β-alanine, L-glutamic acid.

In the presence of L-alanine the optical density consistently gives lower readings as if there occurred a lysis of bacilli in its presence. The greatest difference between *M. tuberculosis* and *M. africanum* was observed when L-alanine is used as the sole N-source.

When 9 more strains each of *M. tuberculosis* were grown in minimal medium with L-alanine as the sole N-source, the difference with *M. africanum* was found to be a constant character. This was also true for *M. bovis* (see table 3).

Some mixtures of amino-acids were also tested (table 2). It appeared that in the case of *M. tuberculosis* and frequently for *M. africanum* growth on L-glutamic and L-aspartic acid was inhibited when L-asparagine was added. It is not known in how far this is a repeatable phenomenon for *M. tuberculosis.*

The addition of L-asparagine to L-alanine did not result in growth inhibition for either *M. bovis*, *M. tuberculosis* or the African strains.

TABLE 3

Effect of L-Ala (5 μmole/ml) on growth of *M. africanum* and *M. tuberculosis*

	Strain n°	% growth	Mean % growth
M. tuberculosis	3463	189	
	3720	379	
	3738	577	
	3761	316	
	3762	334	366
	3764	508	
	4149	191	
	4150	264	
	4152	346	
	4153	454	
M. africanum	2046	46	
	3603	30	
	3605	55	
	3604	50	46
	3608	39	
	3606	50	
	3607	50	
M. bovis	322	50	
	425	45	
	740	55	
	908	60	
	1407	58	60
	1563	73	
	1996	60	
	2014	67	
	3569	75	

Discussion

The taxonomy of *M. africanum* has always been unclear (Boveroulle *et al.*, 1965; Korsak and Millet, 1970; Pattyn *et al.*, 1970).

The growth of tubercle bacilli in the presence of amino-acids was studied by several authors Marshak (1951), Youmans (1954), and Lyon and Hall (1974). Although the techniques applied are widely different and the composition of the basal medium greatly influence the results, all agree that L-asparagine, L-glutamic acid and L-aspartic acid and L-alanine stimulate the growth of *M. tuberculosis*, be it in a different order of importance.

Our results show that if L-alanine is present in a medium as a sole nitrogen source it inhibits the growth of *M. africanum* and *M. bovis* but

not that of *M. tuberculosis.* This adds one character more differentiating *M. africanum* from *M. tuberculosis* and at the same time approaching it to *M. bovis* (Pattyn *et al.*, 1970).

Ujije *et al.* (1967) presented evidence of some amino-acids (asparagine, aspartic acid and serine) inhibiting the growth of dysgonic strains of *M. tuberculosis* in complex media, we could not confirm these results with *M. africanum.* This is however probably the result of the differences in the basal media used.

Samenvatting — **Vergelijkende studie van de invloed van verschillende koolstof en stikstofhoudende produkten op de groei van** *Mycobacterium africanum, tuberculosis* **en** *bovis.*

In een basaal minimum medium, met glucose als koolstofbron, was *M. africanum* niet in staat L-α alanine als enige stikstofbron te gebruiken. *M. africanum* gedraagt zich hierbij zoals *M. bovis* en verschillend van *M. tuberculosis.*

Résumé — **Etude comparative de l'utilisation de différents composés de carbone et d'azote par** *Mycobacterium africanum, tuberculosis* **et** *bovis.*

Dans un milieu de culture minimal avec le glucose comme seule source de carbone, *M. africanum* est incapable d'utiliser la L-α alanine comme seule source d'azote. Dans ces conditions *M. africanum* se comporte comme *M. bovis* et diffère de *M. tuberculosis.*

Bacteriology Department, Institute of Tropical Medicine, Nationalestraat 155 — B-2000 Antwerpen, Belgium.
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