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RESEARCH NOTE

Survival of *Mycobacterium ulcerans* at 37°C

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ABSTRACT

Bone infection and metastatic spread in cases of Buruli ulcer imply that *Mycobacterium ulcerans* is able to survive and multiply at 37°C. This study investigated the survival at 37°C of *M. ulcerans* isolates from diverse geographical and clinical sources. Although the viability of all isolates decreased after a few days at 37°C, viable bacilli remained after 13 days at 37°C in most instances. African isolates of *M. ulcerans* were more thermotolerant than isolates from temperate regions. Isolates from skin and bone lesions of the same patients showed no difference in thermotolerance.

Keywords Buruli ulcer, geographical origin, *Mycobacterium ulcerans*, survival, thermotolerance

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Mycobacterium ulcerans, the causative agent of Buruli ulcer (BU), grows optimally on mycobacteriological media at 30–32°C [1]. *M. ulcerans* classically infects skin and subcutaneous tissue, where the pathogen encounters favourable growth temperatures. However, metastatic spread to distant skin sites or bone also occurs [2–5], suggesting that *M. ulcerans* is able to survive and/or multiply at 37°C. The present study investigated the survival of *M. ulcerans* at 37°C on Löwenstein–Jensen medium. The results may have implications for: (i) a better understanding of the clinical features of the disease, with bone involvement resulting from metastatic spread; (ii) the treatment of lesions with local heat; and (iii) the epidemiology and transmission of the disease in relation to survival of *M. ulcerans* in the environment at high temperatures.

Seventeen isolates of *M. ulcerans* from ten countries were initially studied. Tubes of Löwenstein–Jensen medium were inoculated with serial dilutions of suspensions of bacteria and incubated at 37°C for 0, 3, 6, 9 or 24 h, and 2, 3, 6, 9 or 13 days, and thereafter at 32°C for 12 weeks. The number of surviving bacilli was estimated by counting the number of CFU. Inactivation curves were obtained for each isolate by plotting CFU/mL against exposure time at 37°C on a semi-logarithmic scale. The slope of the inactivation curves was expressed as decimal reduction time (D), measuring heat resistance and the time needed to inactivate 90% of the bacterial population at a given temperature. A higher D value thus indicates a greater thermotolerance [6,7].

Table 1 shows that a decrease in viability occurred for all isolates after a few days at 37°C, but with large variations for individual isolates. However, for all isolates except those from Japan, China and French Guiana, viable bacilli were still present after incubation for 13 days at 37°C. The

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Table 1. Isolates of *Mycobacterium ulcerans* from different geographical or clinical origins showing their decimal reduction times (D values) at 37°C

Strain number	Country of origin	D value (days)	Patient number	Strain number	Origin of specimen	D value (days)
ITM 5142	Australia (Victoria); human tissue (ATCC 19423)	5.74	97-10	ITM 97-0952	Skin	4.28
ITM 95-1112	Australia (Victoria); human tissue	3.18		ITM 97-0684	Bone	7.27
ITM 8756	Japan; human tissue (ATCC 33728)	2.22	98-301	ITM 99-0826	Skin	10.60
ITM 98-0912	China; human tissue	2.17		ITM 99-0742	Bone	5.90
	Average for non-tropical Australasia	**3.33 ± 1.67**	99-20	ITM 99-2654	Skin	3.81
ITM 94-1328	Malaysia; human tissue	7.81		ITM 99–1567	Bone	7.02
ITM 03-0524	Papua New Guinea; human tissue	3.37	99-86	ITM 99-1721	Skin	7.74
	Average for tropical Australasia	**5.59 ± 3.14**		ITM 99-1722	Bone	6.88
ITM 5143	Mexico; human tissue	9.80		ITM 99-1723	Bone	4.47
ITM 842	Surinam; human tissue	3.12	99-226	ITM 99-2949	Skin	7.38
ITM 7922	French Guiana; human tissue	2.31		ITM 00-0040	Bone	4.15
	Average for Latin America	**5.08 ± 4.11**	00-225	ITM 01-0566	Skin	5.18
ITM 95-1009	Togo; human tissue	5.04		ITM 01-0407	Bone	4.31
ITM 00-1441	Benin; aquatic insect	6.15	01-26	ITM 01-0498	Skin	3.73
ITM 96-0658	Angola; human tissue	6.05		ITM 01-0499	Bone	5.59
ITM 02-0279	Cameroon; human tissue	10.55	00-156	ITM 00-0946	Skin	7.48
ITM 03-0216	Benin; human tissue	16.98		ITM 00-0945	Skin	4.14
ITM 03-0221	Democratic Republic of Congo; human tissue	13.04		ITM 00-1034	Skin	7.70
ITM 94-0815	Côte d'Ivoire; human tissue	7.03	99-388	ITM 00-1714	Bone	11.48
ITM 01-1867	Ghana; human tissue	7.40			**Average for bone**	**6.34 ± 2.27**
	Average for Africa	**9.03 ± 4.16**			**Average for skin**	**6.21 ± 2.31**

D values varied between 2.17 days for a Chinese isolate, and 16.98 days for an isolate from Benin (ITM 03-0216). This means that it takes 2.17 days at 37°C to reduce a bacterial population of 1000 CFU of the Chinese isolate to a population of 100 CFU, while the same reduction would take 16.98 days for the isolate from Benin. Thus, the Chinese isolate was much more sensitive to heat inactivation at 37°C than was the isolate from Benin. The average D value of 9.03 days for African isolates was higher than that for isolates from other continents, but this was only significant when compared with isolates from non-tropical countries in Australasia (p 0.027). In non-tropical regions of Australia, China and Japan, BU is complicated only rarely by the osteomyelitis or multifocal forms that are seen in Africa [4,5]. For this reason, African strains might be expected to tolerate prolonged incubation at 37°C. Schulze-Röbbecke and Buchholtz [7] found a D value of 2.33 days at 37°C for a *Mycobacterium marinum* strain from Philadelphia, USA, which is comparable to the values obtained in the present study for isolates from non-tropical regions (average 3.33 days).

In a collection at this laboratory of 944 *M. ulcerans* isolates from worldwide locations, 215 (22.8%) showed growth at 37°C. Of these, 189 (87.9%) isolates yielded more colonies at 30°C, and only 23 (10.7%) showed the same growth at 37°C and 30°C. Interestingly, three (1.5%) African isolates yielded more colonies at 37°C than at 30°C, which is in agreement with the higher thermotolerance of African isolates at 37°C that was observed in the present study. African strains of *M. ulcerans* also show genetic differences as compared with strains from other continents [8–12]. In addition, they are much more virulent in a mouse model (F. Portaels *et al*., unpublished data) and produce a different mycolactone [13].

Nineteen isolates from nine patients in Benin with both bone and skin lesions were also studied (Table 1). There was no difference in thermotolerance among isolates from skin and bone specimens (mean D values of 6.21 vs. 6.34 days, respectively). The disparity between the thermotolerance of *M. ulcerans* to 37°C on Löwenstein–Jensen medium and its resistance to the same temperature in infected bone may be explained by the differences between local conditions in the bone and the bacteriological growth medium.

In view of the efficacy of heat treatment, survival of *M. ulcerans* should also be tested at temperatures >37°C (e.g., 40°C). Meyers *et al*. [14] have demonstrated that a rigorously controlled regimen of local heat therapy at 40°C can be curative for BU. Moreover, it was also found that subsequent growth at 32°C was retarded after exposure of cultures of the bacteria to 37°C for 1 day, and was completely inhibited after exposure to 40°C for 10 days. In a previous study, it was observed that exposure to 41°C for 1 day kills >90% of the bacilli [15].

Tolerance of *M. ulcerans* to moderately elevated temperatures may have implications for the epidemiology of BU in tropical and non-tropical countries. In southern Australia, which has a mild temperate climate, it has been hypothesised that *M. ulcerans* spreads in the environment through aerosols arising from contaminated water, which may, in turn, infect humans through contamination of skin lesions [16]. This seems plausible in the temperate climate of southern Australia, but in tropical areas, such as Africa, temperatures away from water can be sufficiently elevated (>40°C) for *M. ulcerans* not to survive in aerosols.

In conclusion, isolates obtained from skin and bone lesions from the same patients show no difference in thermotolerance, but African isolates of *M. ulcerans* appear to be more thermotolerant than isolates from temperate regions. There was an increase in the D values, from 3.3 days for isolates from non-tropical Australasia to 9.03 days for isolates from Africa. This observation could be linked to the fact that osteomyelitis and multifocal lesions occur in Africa, but almost never occur in non-tropical Australasia.

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