Analysis of the *Mycobacterium ulcerans* genome sequence reveals new loci for variable number tandem repeats (VNTR) typing

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Screening of the genome sequence of the *Mycobacterium ulcerans* strain Agy99 from Ghana with tandem repeats finder software revealed 34 novel non-degenerate tandem repeats containing loci suitable for variable number tandem repeats (VNTR) typing. All loci revealed polymorphism within *M. ulcerans* isolates of geographically diverse origins. The results confirm the evolutionary scenario suggested by multi-locus sequence typing in which a progenitor of all *M. ulcerans* lineages emerged from the environmental species *Mycobacterium marinum* and subsequently diverged into several geographical lineages. For further attempts to develop a VNTR-based genetic fingerprinting tool for *M. ulcerans*, it is suggested that the focus should rather be on *M. marinum* than on the African *M. ulcerans* Agy99 genome sequence as a starting point.

INTRODUCTION

Mycobacterium ulcerans, a slow-growing environmental mycobacterium (Yeboah-Manu *et al.*, 2004), causes Buruli ulcer, which is, after tuberculosis and leprosy, the third most important mycobacterial disease worldwide (Sizaïre *et al.*, 2006; van der Werf *et al.*, 2003). Buruli ulcer is mainly prevalent in West Africa but cases have also been reported from various other tropical and sub-tropical areas of the world (Asiedu *et al.*, 2000; Johnson *et al.*, 2005). Genetic analysis indicates the emergence of *M. ulcerans* from *M. marinum*, a fish pathogen which can also cause disease in humans (Boddinghaus *et al.*, 1990; Tonjum *et al.*, 1998). There is a strong association between the occurrence of *M. ulcerans* infection in humans and slow-flowing or stagnant water bodies (Marsollier *et al.*, 2007; Portaels *et al.*, 1999; Roberts & Hirst, 1997; Ross *et al.*, 1997; Stinear *et al.*, 2000a). Aquatic snails (Marsollier *et al.*, 2004), fish (Eddyani *et al.*, 2004) and water bugs (Marsollier *et al.*, 2002) are currently being discussed as passive and active hosts, respectively. However, the exact mode of transmission of Buruli ulcer has remained a mystery, partly because no genetic fingerprinting tool is available for micro-epidemiological analysis of infection chains.

Variable number tandem repeats (VNTR) typing is a promising typing tool for *M. ulcerans*, because analysis based on few loci already has a higher discriminatory power

than other standard molecular typing methods (Ablordey *et al.*, 2005a, b; Hilty *et al.*, 2006; Stragier *et al.*, 2005, 2006). VNTR typing thus revealed for the first time genetic diversity within isolates from African countries (Hilty *et al.*, 2006; Stragier *et al.*, 2006). So far, two approaches have been used to identify loci suitable for VNTR typing of *M. ulcerans*. These are the screening of the *Mycobacterium marinum* genome sequence for tandem repeats containing loci with tandem repeats finder software (Ablordey *et al.*, 2005b; Hilty *et al.*, 2006), and BLAST searches for homologues of *M. tuberculosis* mycobacterial interspersed repetitive units in *M. ulcerans* (Stragier *et al.*, 2005). Here, we have searched for the first time the completely assembled *M. ulcerans* genome sequence for tandem repeats containing loci and have evaluated the potential of the identified loci for VNTR-based typing.

METHODS

Identification of the tandem-repeat-containing loci. Tandem repeats finder software (<http://tandem.bu.edu/trf/trf.html>) was used to screen the *M. ulcerans* Agy99 sequence database and to identify tandem-repeat-containing loci. Selection criteria were a repeat size of >20 bp, a perfect (100%) sequence identity of the repeats and an entropy of repeat sequences >1.70 within the total entropy range of 0–2 to allow for easy determination of the number of repeats by agarose gel electrophoresis and to exclude very similar (e.g. GC-rich) sequences which do not represent tandem repeats.

Primer design. Specific primers targeting repeat-flanking genomic sequences of *M. ulcerans* Agy99 were designed using Frodo Primer 3 software (<http://frodo.wi.mit.edu/>). According to sequence information from the *M. marinum* genome database (www.sanger.ac.uk/projects/M_marinum/), the *M. marinum* sequences corresponding to

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Abbreviation: VNTR, variable number tandem repeats.

Table 1. Newly identified VNTR loci and primers used for VNTR typing

Repeat positions and sizes, primer sequences and PCR product sizes were determined from the reverse complement genome sequence of *M. ulcerans* strain Agy99 published recently (Stinear *et al.*, 2007). Primers which had single nucleotide differences in the *M. marinum* target sequence are indicated in bold (primer no.).

VNTR locus no.	Position in Agy99 genome	Repeat sequence (repeat size)	Size of PCR product (Agy99)	Primer no.	Primer sequence
1	75 046	AGAAGCCGAGCTGACGCGACGCGGCCAACCTTCAAA (37)	232	1579	CATAGCCACGTGACCAATGT
				1580	GTGAGCAGGGCATCTACCAG
2	260 371	ATGCCACGGTGGGCAATCCCTACCCCG (27)	238	1671	TGGTGTGGTTGCGTCTTCT
				1672	GGACAAAGACGCGAGAAAGT
3	624 989	CTGTTCAAGGGATTCAATGTGGGCCTCG (28)	236	1623	AAGATCATTCTGCCGCTCAT
				1624	CACAACCGAAGATCAACACG
4	688 860	AGTTCCTCTCGCCAGGCATGGATGC (25)	185*	1673	CGTTGTGACACGTCATCCAT
				1674	GTTCCGGTCTGCGATACTGT
5	719 016	TCTTCTCCTTCGACTTGGGCAGGCGTC (27)	362	1625	CAACCTCGTCGAGATCAACC
				1626	AAGGTGACGCAAGCAATGTT
6	759 207	TGCCGAAATCAGCCGACGGCGCAGGCCAGCTGGCGGC-CGTCGCCGGCGGCCAAGATCCGGTGACACTGAACCTCGA-TGGGTTGGGCCGCTACCGGGTGGTGTCCGCCCTGAGCCG-CCGCGGTGACGAACTCATCGTCACCGGCCTTTTCGA (152)	388	1581	GCGGGGTATCTGACCAATAG
				1582	GTGACGATGCCGAAAATGAT
7	898 478	TCCTTTTAGAACCCTCCAAGCGCG (24)	232	1675	CTTTCTCCATGCGGGAGTT
				1676	TCGTTGCTGTCACCAGACTC
8	1 023 923	CGCCCGCTGGTGAGGCCGTTCCGGACGAAC (30)	304	1583	CTTCCCATCAATGGTTTTTG
				1584	GCCCGGTGAACCAACTACTA
9	1 440 525	GCCACCCTACGGACCAATTAC (21)	160	1679	CGCACTGGTGAGTCTGGATA
				1680	GGCATCCAACCTGACTGACA
10	1 563 743	CGAGCTGTGTTCTGCTGGTGCGCCAT (26)	278	1627	CATCGCCACAAGTGTTTTCA
				1628	TTCGGTTCCTTTCACATGGT
11	1 773 797	CACCCTTTGCCCAACACTTGC (21)	194	1681	AAACGACATCGACCAGACG
				1682	CCGAGTCGAAGTTGGTATGC
12	1 973 523	GTCGGCACCGATGAGGTAATCG (22)	189	1683	CCGCATACGATCTGTTCAGT
				1684	ACCGAAAGTCCAGGGTTCTT
13	2 018 640	GCCACATATGGCAACCCCTTGGGGATAC (28)	317*	1591	AACTCCGGGATTGGTTCTTT
				1592	AACGTCGTCTGTTTCGGTTC
14	2 314 530	GAATGCCGTGTCTTCACAACTGCCG (26)	325	1629	GACCGTAAACCGACGAAAAA
				1630	CACTAATATGCGGTGCGTTC
15	2 475 473	CGACACCCGGTATTCCCGCATAT (23)	228	1685	CCGTAGATGGTCTGGATGC
				1686	CCCGAACCTATCTCGATGAC
16	2 549 331	CGCATTGCGAAGTGCGGACG (21)	352	1631	ATGCAACCACTTGCCCATC
				1632	AGCAGTTCTTCGGTGACGAC

Table 1. cont.

VNTR locus no.	Position in Agy99 genome	Repeat sequence (repeat size)	Size of PCR product (Agy99)	Primer no.	Primer sequence
17	2 847 974	AGACTCCGGAGTTCATGGCCATTCGTC (27)	304	1585	GTTATCACCCGATTGCCAGT
18	3 474 730	ACCTCGGCGGTACCCGCGAACAG (28)	250	1586	AAACAACGACTTTCGCGTCT
19	3 709 194	TGATCTCGCCTTCCTCGTTACCCACCGG (28)	224	1587	AGGTCGACTTCGTGCTCAAC
20	3 802 904	ACCGATCTATTGGCCGCAAAG (21)	250	1588	ATGGGCGTAGGTCCAAAAT
21	3 913 042	ACCATTGCCGAGCTGGTGAAT (21)	279	1589	AGGATCGCCTTCTTGTTTCC
22	4 062 301	ATGAATTCTTGCGAGATTCTT (21)	313	1590	TGCAGTGCATCGTTCATACC
23	4 110 344	AAATCGTCACGCCGGAAGTT (21)	182	1687	TGAGTTGGCCCAGTCGAG
24	4 145 439	CAGTCATCGGAATTCCTCCCAATC (23)	315	1688	GACAAGCCGGCCAAACATC
25	4 251 686	AACCTGCCCTGGTCGTCGGAGTT (23)	355	1635	GTCACCGTCAACATCACGTC
26	4 450 491	CGAGCGCAGGTGCACATGGTG (21)	193†	1636	GGTCAGCACGTTTTTCATGC
27	4 559 395	TTCAGTCAGGGGCTGAGCAAC (21)	150	1637	GTATGCGCGACTGATCCAC
28	4 596 959	CGGCTTTCAAATCGTTTTGTGCGGTCGAC (28)	324	1638	CCGTTTGGCTATCCGATAAA
29	4 837 553	CTATGGCGACACCTACACCGA (21)	231	1691	GGACCGACTGTTGATGGTTT
30	4 964 512	CGCCCAGAACAGCCGTCATAC (21)	211	1692	TGGAGTCCAGGAAGAACTCG
31	5 006 187	CGGGTCGCCGGCCTCACATCT (21)	310	1639	GAAGCCCATCGTGTCTGC
32	5 135 404	GCTCCGCTGCCTAGGTTTCGAC (21)	344	1640	CTTACCTGGGCGATGAGTGT
33	5 210 577	GGTTGTCCAGCCGCGCGCTGA (24)	240	1641	CAGCAGGTCCGCTATGAAGT
34	5 323 208	ATCAACTATCTGTGCGAGACCGGTGCGTT (29)	301	1642	GTCGTTGAAGGGGTGACACT
				1693	TTGCCGAAATTTCTCTTTGG
				1694	AGTCGGTCAGGGGTGGT
				1695	TGAGCCGATAGTGCAAAAGTG
				1696	TCGGTTTGGATGAATTGGTT
				1593	TCTACCCCAACACCGAGAAC
				1594	ATCCAAAATGGACACCGAAG
				1697	TGATTTCCCAAGACGAGAACC
				1698	CCGTTTCGTGTCGCAAGTTGTA
				1699	ACTTACCAACCGCTTTCAC
				1700	ACCAACTCCAAGGTCAACCAG
				1643	GTCAGGGCTTTGATCTCACC
				1644	CGCTGCACGTCAAACTAAG
				1645	CCAGTTGCTGCAGAAGTTCA
				1646	CGAAGTGAAAGGTGCGTTTGG
				1701	GTGTTTCAGCAGGTTGGACTG
				1702	TCTGATGACCCATGACCTGA
				1597	TGCTGCTGGATTGTTTTTCG
				1598	CATGATGCCAAGCAGCAC

*French Guyana isolate did not amplify.

†Ghana ITM-970359 did not amplify.

these primers were 100 % identical, except for four primers, 1676, 1682, 1684 and 1692, which contained single nucleotide mismatches (Table 1).

PCR analysis and copy number designation. A panel consisting of 11 *M. ulcerans* clinical isolates of human origin from diverse geographical areas was used to assess the polymorphism of the VNTR loci. PCR for tandem-repeat-containing loci (Table 1) was performed as described by Hilty *et al.* (2006). Briefly, PCR mixtures (final volume, 20 µl) contained 2 µl *Taq* PCR buffer, deoxynucleoside triphosphates (0.2 mM each), 1 U *AmpliTaq* DNA polymerase (Perkin-Elmer Applied Biosystems), each primer at a concentration of 0.5 µM and mycobacterial DNA. PCR was performed for 40 cycles of 0.5 min at 94 °C, 0.5 min at 65 °C and 1 min at 72 °C. The reactions were terminated by incubation for 10 min at 72 °C.

PCR fragments were analysed by agarose gel electrophoresis using 2 % NuSieve agarose, and sizes of the amplicons were estimated by comparison with Size Marker VIII (Roche), compared with the product size of Agy99 and copy number designated.

RESULTS AND DISCUSSION

Screening of the *M. ulcerans* strain Agy99 genome sequence from Ghana with tandem repeats finder software identified 45 perfect tandem-repeat-containing loci which have not been described in previous studies (Ablordey *et al.*, 2005a, b; Hilty *et al.*, 2006; Stragier *et al.*, 2005, 2006). Eleven out of 45 loci were excluded from further analysis for various reasons, including the presence of insertion sequence elements. For the remaining 34 loci, primers were designed (Table 1) and PCR amplification of a panel of 11 well-characterized *M. ulcerans* clinical isolates of diverse geographical origins performed. As it was our primary objective to develop a genetic fingerprinting method for African *M. ulcerans* isolates, more strains from Africa (6) than from other continents were analysed.

All 34 loci showed polymorphisms within the 11 isolates tested, but only alleles with one and with two repeats were

found. Based on the typing results, the new VNTR loci were classified into four different subgroups, designated i–iv (Table 2). While subgroups i and ii comprised a multitude of loci (17 and 15, respectively), subgroups iii and iv were each represented by only one locus. For all 34 new VNTR loci, the *M. marinum* genome sequencing strain and the *M. ulcerans* isolates from Japan and French Guyana had only one repeat (genotype A; Table 2). In contrast, all six African *M. ulcerans* isolates analysed plus strain Agy99 from Ghana had two repeats in all 34 loci (VNTR genotype E; Table 2), indicating that they have further diverged from *M. marinum* than the other tested geographical lineages of *M. ulcerans*. The isolates from Australia, Malaysia and Papua New Guinea had one repeat in all 17 subgroup i loci and two repeats in all 15 subgroup ii loci, thus sharing a comparable number of alleles with both the *M. marinum*-associated VNTR genotype A and the African *M. ulcerans*-associated genotype E. They could be further subdivided into three VNTR genotypes (designated B–D) by differences in the two VNTR loci 15 and 24 constituting subgroups iii and iv (Table 2). These results indicate an evolutionary scenario in which a progenitor of all *M. ulcerans* lineages emerged from *M. marinum*, subsequently diverging into several geographical lineages. The African lineage may have evolved from an ancestral lineage from the Western Pacific region (Australia, Malaysia and Papua New Guinea) which may itself have a progenitor in Asia. These results may confirm the evolutionary scenario suggested by multi-locus sequence typing (Stinear *et al.*, 2000b).

In this study, our selection criteria for finding tandem-repeat-containing loci were highly stringent and it should be possible to identify more VNTR loci, such as degenerate-repeat-containing loci and loci with repeat sizes of <20 bp. While for all 34 new loci identified here by screening of the *M. ulcerans* Agy99 genome sequence only alleles with one and with two repeats were found, Ablordey *et al.* (2005b)

Table 2. VNTR genotypes defined by the 34 new VNTR loci

VNTR genotype	Origin of strains*	No. of tandem repeats in the subgroups of new VNTR loci†			
		i (17 loci)	ii (15 loci)	iii (1 locus)	iv (1 locus)
A	Japan, French Guyana, <i>M. marinum</i>	1	1	1	1
B	Australia	1	2	1	1
C	Malaysia	1	2	2	1
D	Papua New Guinea	1	2	2	2
E	Africa	2	2	2	2

*Japan, ITM-8756; French Guyana, ITM-7922; *M. marinum*, derived from the *M. marinum* genome sequence (www.sanger.ac.uk/projects/M_marinum); Australia, ITM-884; Malaysia, ITM-941328; Papua New Guinea, ITM-9357. African isolates: Ghana, ITM-970321 and ITM-970359; Benin, ITM-940512 and ITM-940886; Ivory Coast, ITM-940662; Angola, ITM-960658; and Agy99 (derived from <http://genopole.pasteur.fr/Mulc/BuruList.html>).

†Subgroup i, VNTR 1, 6, 7, 8, 9, 11, 12, 14, 18, 19, 21, 23, 26, 29, 30, 31 and 34; subgroup ii, 2, 3, 4, 5, 10, 13, 16, 17, 20, 22, 25, 27, 28, 32 and 33; subgroup iii, VNTR 15; subgroup iv, VNTR 24.

also described alleles with more than two repeats for 6/9 VNTR loci identified by screening the *M. marinum* genome. *M. marinum* genome-assisted identification of new VNTRs has led to the first demonstration of genetic diversity in the same panel of African *M. ulcerans* isolates as used in this study (Hilty *et al.*, 2006). In contrast, the strain Agy99 genome-based approach used here did not identify another VNTR locus that differentiates African isolates. Therefore, attempts to refine VNTR-based genetic fingerprinting for micro-epidemiological analysis of *M. ulcerans* should rather focus on the *M. marinum* than on the *M. ulcerans* Agy99 genome sequence as a starting point.

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REFERENCES

- Ablordey, A., Hilty, M., Stragier, P., Swings, J. & Portaels, F. (2005a). Comparative nucleotide sequence analysis of polymorphic variable-number tandem-repeat loci in *Mycobacterium ulcerans*. *J Clin Microbiol* **43**, 5281–5284.
- Ablordey, A., Swings, J., Hubans, C., Chemlal, K., Loch, C., Portaels, F. & Supply, P. (2005b). Multilocus variable-number tandem repeat typing of *Mycobacterium ulcerans*. *J Clin Microbiol* **43**, 1546–1551.
- Asiedu, K., Scherpier, R. & Raviglione, M. (2000). *Buruli Ulcer – Mycobacterium ulcerans Infection*. WHO Document, WHO/CDS/CPE/BG/0001.
- Boddinghaus, B., Rogall, T., Flohr, T., Blocker, H. & Bottger, E. C. (1990). Detection and identification of *Mycobacteria* by amplification of ribosomal-RNA. *J Clin Microbiol* **28**, 1751–1759.
- Eddyani, M., Ofori-Adjei, D., Teugels, G., De Weirde, D., Boakye, D., Meyers, W. M. & Portaels, F. (2004). Potential role for fish in transmission of *Mycobacterium ulcerans* disease (Buruli ulcer): an environmental study. *Appl Environ Microbiol* **70**, 5679–5681.
- Hilty, M., Yeboah-Manu, D., Boakye, D., Mensah-Quainoo, E., Rondini, S., Schelling, E., Ofori-Adjei, D., Portaels, F., Zinsstag, J. & Pluschke, G. (2006). Genetic diversity in *Mycobacterium ulcerans* isolates from Ghana revealed by a newly identified locus containing a variable number of tandem repeats. *J Bacteriol* **188**, 1462–1465.
- Johnson, P. D., Stinear, T., Small, P. L., Pluschke, G., Merritt, R. W., Portaels, F., Huygen, K., Hayman, J. A. & Asiedu, K. (2005). Buruli ulcer (*M. ulcerans* infection): new insights, new hope for disease control. *PLoS Med* **2**, e108.
- Marsollier, L., Robert, R., Aubry, J., Saint Andre, J. P., Kouakou, H., Legras, P., Manceau, A. L., Mahaza, C. & Carbonnelle, B. (2002). Aquatic insects as a vector for *Mycobacterium ulcerans*. *Appl Environ Microbiol* **68**, 4623–4628.
- Marsollier, L., Severin, T., Aubry, J., Merritt, R. W., Saint Andre, J. P., Legras, P., Manceau, A. L., Chauty, A., Carbonnelle, B. & Cole, S. T. (2004). Aquatic snails, passive hosts of *Mycobacterium ulcerans*. *Appl Environ Microbiol* **70**, 6296–6298.
- Marsollier, L., Andre, J. P., Frigui, W., Reyssset, G., Milon, G., Carbonnelle, B., Aubry, J. & Cole, S. T. (2007). Early trafficking events of *Mycobacterium ulcerans* within *Naucoris cimicoides*. *Cell Microbiol* **9**, 347–355.
- Portaels, F., Elsen, P., Guimaraes-Peres, A., Fonteyne, P. A. & Meyers, W. M. (1999). Insects in the transmission of *Mycobacterium ulcerans* infection. *Lancet* **353**, 986.
- Roberts, B. & Hirst, R. (1997). Immunomagnetic separation and PCR for detection of *Mycobacterium ulcerans*. *J Clin Microbiol* **35**, 2709–2711.
- Ross, B. C., Marino, L., Oppedisano, F., Edwards, R., Robins-Browne, R. M. & Johnson, P. D. (1997). Development of a PCR assay for rapid diagnosis of *Mycobacterium ulcerans* infection. *J Clin Microbiol* **35**, 1696–1700.
- Sizaire, V., Nackers, F., Comte, E. & Portaels, F. (2006). *Mycobacterium ulcerans* infection: control, diagnosis, and treatment. *Lancet Infect Dis* **6**, 288–296.
- Stinear, T., Davies, J. K., Jenkin, G. A., Hayman, J. A., Oppedisano, F. & Johnson, P. D. (2000a). Identification of *Mycobacterium ulcerans* in the environment from regions in Southeast Australia in which it is endemic with sequence capture-PCR. *Appl Environ Microbiol* **66**, 3206–3213.
- Stinear, T. P., Jenkin, G. A., Johnson, P. D. R. & Davies, J. K. (2000b). Comparative genetic analysis of *Mycobacterium ulcerans* and *Mycobacterium marinum* reveals evidence of recent divergence. *J Bacteriol* **182**, 6322–6330.
- Stinear, T. P., Seemann, T., Pidot, S., Frigui, W., Reyssset, G., Garnier, T., Meurice, G., Simon, D., Bouchier, C. & other authors (2007). Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer. *Genome Res* **17**, 192–200.
- Stragier, P., Ablordey, A., Meyers, W. M. & Portaels, F. (2005). Genotyping *Mycobacterium ulcerans* and *Mycobacterium marinum* by using mycobacterial interspersed repetitive units. *J Bacteriol* **187**, 1639–1647.
- Stragier, P., Ablordey, A., Bayonne, L. M., Lugor, Y. L., Sindani, I. S., Suykerbuyk, P., Wabinga, H., Meyers, W. M. & Portaels, F. (2006). Heterogeneity among *Mycobacterium ulcerans* isolates from Africa. *Emerg Infect Dis* **12**, 844–847.
- Tonjum, T., Welty, D. B., Jantzen, E. & Small, P. L. (1998). Differentiation of *Mycobacterium ulcerans*, *M. marinum*, and *M. haemophilum*: mapping of their relationships to *M. tuberculosis* by fatty acid profile analysis, DNA–DNA hybridization, and 16S rRNA gene sequence analysis. *J Clin Microbiol* **36**, 918–925.
- van der Werf, T. S., Stinear, T., Stienstra, Y., van der Graaf, W. T. A. & Small, P. L. (2003). Mycolactones and *Mycobacterium ulcerans* disease. *Lancet* **362**, 1062–1064.
- Yeboah-Manu, D., Bodmer, T., Mensah-Quainoo, E., Owusu, S., Ofori-Adjei, D. & Pluschke, G. (2004). Evaluation of decontamination methods and growth media for primary isolation of *Mycobacterium ulcerans* from surgical specimens. *J Clin Microbiol* **42**, 5875–5876.

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