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Review

The genome, evolution and diversity of *Mycobacterium ulcerans*

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ABSTRACT

Mycobacterium ulcerans (*M. ulcerans*) causes a devastating infection of the skin and underlying tissue commonly known as Buruli ulcer (BU). Genetic analyses indicate that *M. ulcerans* has a common ancestor with *Mycobacterium marinum* (*M. marinum*) and has diverged from this fish and human pathogen perhaps around a million years ago. *M. ulcerans* is characterized by minimal genetic diversity and since it has a highly clonal population structure, genetic differences between individual isolates reflect changes that have occurred sequentially from their respective progenitors. This feature, which is shared by other bacterial pathogens with low sequence diversity, such as *Yersinia pestis* and *Bordetella pertussis* renders *M. ulcerans* a promising model to reveal evolutionary mechanisms. Until today transmission pathways and environmental reservoirs of *M. ulcerans* are not entirely explored. However, comparative genome analysis of closely related *M. ulcerans* isolates is anticipated to give deeper insights into the population structure of this enigmatic mycobacterium.

Contents

1. Introduction

The genus *Mycobacterium* comprises more than 120 species, many of which are important pathogens of animals and humans (Tortoli, 2006; Zakham et al., 2011). One species within this genus, *Mycobacterium ulcerans* (*M. ulcerans*), is the causative agent of Buruli ulcer (BU), a chronic, necrotizing infection of subcutaneous tissue. BU is reported from more than 30 countries worldwide, but predominantly affects impoverished populations living in remote areas of West Africa (Johnson et al., 2005). One hallmark of the evolution of *M. ulcerans* is the acquisition of a 174 kb virulence plasmid, referred to as pMUM001. This plasmid harbors genes required for the synthesis of the macrocyclic polyketide toxin mycolactone (George et al., 1999), which plays a key role in the pathogenesis of BU. While polyketides are produced by various bacteria, mycolactones are the first identified polyketide virulence determinants of a human bacterial pathogen (Hong et al., 2005a; Rohr, 2000). The cytotoxic and immunosuppressive activities of mycolactone are responsible for the formation of chronic ulcerative skin lesions with only limited inflammatory response in the center of the lesions (Hong et al., 2008). Mycolactone production was initially assumed to be restricted to *M. ulcerans*, but recent studies revealed the existence of other closely related mycolactone-producing mycobacteria (MPM) (Ranger et al., 2006; Rhodes

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et al., 2005; Stragier et al., 2008; Trott et al., 2004). *M. ulcerans* and other MPM strains from around the world are characterized by limited genetic diversity, which is typically found in bacterial populations that have recently gone through an evolutionary bottleneck (Achtman, 2008). This feature may be related to the acquisition of the mycolactone plasmid and adaptation to a new lifestyle. Also other genomic signatures, like proliferation of the insertion sequence elements (ISEs) IS*2404* and IS*2606*, extensive gene loss through pseudogene formation and DNA deletion are suggestive of a bacterial population that has been transformed by DNA acquisition and is adapting to a new, perhaps protected environment, where genes once needed for survival under diverse conditions are no longer required (Stinear et al., 2007).

Although BU is commonly associated with proximity to aquatic habitats, an environmental reservoir of *M. ulcerans* is yet to be identified and so it is not clear to what conditions *M. ulcerans* is adapting. Consequently, the mode of BU transmission remains an enigma. A recent study in South-Eastern Australia, which is the only non-tropical setting reporting significant numbers of BU cases, has implicated tree-dwelling native possums as a possible animal reservoir (Fyfe et al., 2010) and mosquitoes as potential vectors of *M. ulcerans* (Johnson, 2009; Lavender et al., 2011; Merritt et al., 2010). In African BU endemic areas numerous studies focusing on surveys of aquatic environments have suggested the potential of aquatic invertebrate and vertebrate species as well as aquatic vegetation as reservoirs and/or vectors of *M. ulcerans* but definitive evidence is lacking (Kotlowski et al., 2004; Marsollier et al., 2002, 2007; Merritt et al., 2005; Portaels et al., 1999, 2008; Williamson et al., 2008). Since routine methods for the cultivation of the extremely slow growing *M. ulcerans* from environmental sources are not available, these studies are largely based on the detection of *M. ulcerans* genomic DNA sequences by PCR. However, this approach is complicated by the presence of the target DNA sequences used in other MPM. Furthermore, only one *M. ulcerans* strain has ever been isolated from a non-vertebrate source (Portaels et al., 2008). Hence, the relevance of PCR positive environmental samples for the transmission of *M. ulcerans* to humans is not clear.

Prior to the application of high-throughput genome sequencing, high-resolution genetic fingerprinting methods for *M. ulcerans* suitable for micro-epidemiological studies in BU endemic areas could not be developed due to the distinctive genetic monomorphism of *M. ulcerans* strains within a BU endemic region. Comparative genome sequencing has now enabled a genome-wide search for single nucleotide polymorphisms (SNPs). High-resolution SNP typing allows for a differentiation of *M. ulcerans* disease isolates from BU endemic areas into sets of haplotypes. A first retrospective study using this approach has revealed a focal transmission pattern of certain haplotypes (Röltgen et al., 2010), and future longitudinal micro-epidemiological studies involving SNP typing are anticipated to provide deeper insight into *M. ulcerans* transmission pathways and relevant reservoirs.

2. Emergence of *M. ulcerans* from *M. marinum*

Comparison of partial DNA sequences, commonly used for the identification and differentiation of mycobacteria, has revealed a close genetic relationship between *M. ulcerans* and *M. marinum* long before whole-genome sequences became available. In the highly conserved 16S ribosomal RNA (rRNA) gene *M. ulcerans* and *M. marinum* isolates differ only at one dimorphic position (Portaels et al., 1996; Tønjum et al., 1998) and no difference was found in the 16S–23S rDNA internal transcribed spacer region (Roth et al., 1998). Analysis of eight housekeeping and structural genes revealed average nucleotide sequence identities of >98% between *M. ulcerans* and *M. marinum* (Stinear et al., 2000b). Genomic subtraction analyses have shown, that the major genetic acquisition by *M. ulcerans* was the polyketide synthase locus (Jenkin et al., 2003) harbored in the virulence plasmid pMUM001 (Stinear et al., 2004). Comparative analyses in geographically diverse strains of *M. ulcerans* have demonstrated a common evolutionary origin of their pMUM plasmids (Stinear et al., 2005a). Multi-locus sequence typing (MLST) of a panel of *M. ulcerans*, other MPM and *M. marinum* isolates has indicated that all MPM are genetically closely related. In the same study DNA–DNA hybridization analysis between MPM and *M. ulcerans* strains from Africa, Australia and China unveiled overall mean relative binding ratios of 98%, while mean ratios between MPM and non-MPM strains were only 40% (Yip et al., 2007). These data suggest that MPM have evolved from a common *M. marinum* ancestor (Yip et al., 2007) and comply with proposed criteria for species delineation (Vandamme et al., 1996). Therefore, it is currently debated whether this genetically coherent group of strains should be reclassified as *M. ulcerans* (Pidot et al., 2010a). MLST has shown that the ongoing process of evolution among MPM has generated at least two lineages, which occupy different ecological niches. One lineage includes strains, which typically cause disease in ectotherms such as frogs and fish, while strains of the other lineage cause BU in humans and can infect other endotherms such as possums (Yip et al., 2007). *M. ulcerans* isolates from BU patients can be distinguished from other MPM by the analysis of large sequence polymorphisms (LSPs) (Käser et al., 2009a) and by variable number of tandem repeat (VNTR) typing (Stragier et al., 2007).

Detailed analyses of LSPs between *M. ulcerans* isolates from BU patients of a world-wide origin revealed an intra-species evolutionary scenario with two distinct phylogenetic lineages (Käser et al., 2007; Rondini et al., 2007). *M. ulcerans* haplotypes from Asia, South America and Mexico belong to the ancestral lineage, whereas the classical lineage includes haplotypes from Africa, Australia and South East Asia (Käser et al., 2007). While BU disease caused by strains belonging to the ancestral lineage is only sporadically reported (Faber et al., 2000; Guerra et al., 2008; Nakanaga et al., 2011), focal prevalence of BU in areas of Africa and Australia endemic for disease caused by the classical lineage is much higher. This may indicate that the ancestral lineage, which is genetically closer to *M. marinum* (Käser et al., 2007), is less virulent, but not necessarily less pathogenic, than the classical lineage (Käser et al., 2007; Käser and Pluschke, 2008; Mve-Obiang et al., 2003).

In order to estimate the divergence time of the two *M. ulcerans* lineages, a systematic analysis of synonymous SNPs between African classical lineage isolates and a Japanese isolate belonging to the ancestral lineage has been carried out. Based on a universal clock rate (Ochman et al., 1999), it was estimated that the classical lineage diverged about 400,000 years ago from the ancestral lineage (Qi et al., 2009). Data furthermore suggested that the African strains and *M. marinum* strain M diverged from a common ancestor about 1.1–1.5 million years ago (Fig. 1). However there are many uncertainties associated with such estimates and the major underlying assumption here – that all strains have been evolving at equal rates – needs to be verified.

3. Genome characteristics and reductive evolution

The first complete genome sequence of *M. ulcerans* was from a clinical isolate, called Agy99, isolated in Ghana in 1999 from a BU lesion. The *M. ulcerans* Agy99 genome is composed of a 5631,606-bp chromosome with the 174,155-bp virulence plasmid pMUM001 (Stinear et al., 2007). The chromosome of *M. ulcerans* Agy99 was shown to harbor 4160 protein-coding genes as well as 771 pseudogenes (Stinear et al., 2007). It is rich in insertion