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ARTICLE

Comprehensive review of *Mycobacterium ulcerans* and Buruli ulcer from a bioinformatics perspective – what have we learnt?

Saubashya Sur* and Biswajit Pal

Postgraduate Department of Botany, Life Sciences Block, Ramananda College, Bishnupur-722122, West Bengal, India

ABSTRACT *Mycobacterium ulcerans* is a non-tuberculous mycobacterium responsible for causing Buruli ulcer. This is a neglected tropical disease characterized by ulceration, necrotization and scarring of the soft tissues in human limbs. Pathogenesis of *M. ulcerans* is mediated by a cytotoxic and immunosuppressive compound called mycolactone. This steadily evolving mycobacteria has adapted itself with the aquatic insect ecosystem. Human communities in wetland ecosystems are prone to Buruli ulcer and several endemic regions have been identified. So far, there is no vaccine and surgery or prolonged treatment with antibiotic cocktail has been mandated to overcome resistance patterns. Application of bioinformatics tools in *M. ulcerans* and Buruli ulcer research during the post genomic era, has provided immense opportunities. In this review, we summarize the outcome of genome studies, comparative genomics, population genomics, genetic diversity analysis, phylogenetic studies and proteomics research pertaining to this disease. We also highlight the implications of in silico vaccine design and computational studies on natural products. Resultant findings are conducive for interpreting genome architecture, pathogenomic evolution and intraspecific divergence due to phylogeographic and virulence factors of *M. ulcerans*. Moreover, the outcome of population genomics studies in disease management, coupled with the efforts in discovering vaccine candidates and novel lead compounds, will enrich our understanding of Buruli ulcer.

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***Corresponding author**
E-mail: saubashya@gmail.com

Introduction

The broadly diverse mycobacteria are known to exist in wide ranging environments like soil, water, dust etc. (Eddyani et al. 2008; Johansen et al. 2020). They have been divided into tuberculous and non-tuberculous mycobacteria (NTM) (Wolinsky 1992). Non-tuberculous mycobacteria (NTM) consist of more than 170 different species (Baldwin et al. 2019). While majority of them have a worldwide presence, some are endemic to certain geographical locations (Hoefsloot et al. 2013). NTM are responsible for a wide array of pulmonary, extrapulmonary and disseminated diseases affecting all organs (Baldwin et al. 2019; Johansen et al. 2020). NTM infections are increasing globally owing to a multitude of factors (Collins 1989; Faverio et al. 2016). Constrained diagnostic capabilities, coupled with high degree of antibiotic resistance and lack of vaccines has aggravated the problem (Ahmed et al. 2020).

First identified in 1947, *Mycobacterium ulcerans* is a pathogenic NTM responsible for causing Buruli ulcer (Yotsu et al. 2015). *M. ulcerans* is a fragile (Eddyani et al. 2008) environmental bacterial pathogen (Ohtsuka et al. 2014), that has an optimal growth temperature of 30-33°C (Hoxmeier 2014). It has been known to adapt to certain ecological niches (Stinear et al. 2007) and are transmitted by some aquatic insects, mosquitoes, mammals etc. (Stinear et al. 2004; Einarsdottir and Huygen 2011). Evidences show that *M. ulcerans* evolved one million years ago by diverging from the common ancestor shared by *Mycobacterium marinum* (Stinear et al. 2005). This was made possible by acquisition of a plasmid encoding mycolactone and reductive evolution (Demangel et al. 2009; Hoxmeier 2014). Mycolactone is responsible for the virulence of *M. ulcerans* (Liu et al. 2019). *M. ulcerans* infection results in tissue destruction of limbs owing to necrosis of the skin and soft tissues (Einarsdottir and Huygen 2011; O'Brien et al. 2019).

The World Health Organization (WHO) considers Buruli ulcer as a neglected tropical disease. It has been reported from 34 countries (Fig. 1), especially Ghana, Benin, Democratic Republic of Congo, Ivory Coast, Togo, French Guiana, Australia, China, Papua New Guinea, and Japan (Yotsu et al. 2015; Röltgen and Pluschke 2015, Simpson et al. 2019). People living near swamps, lakes

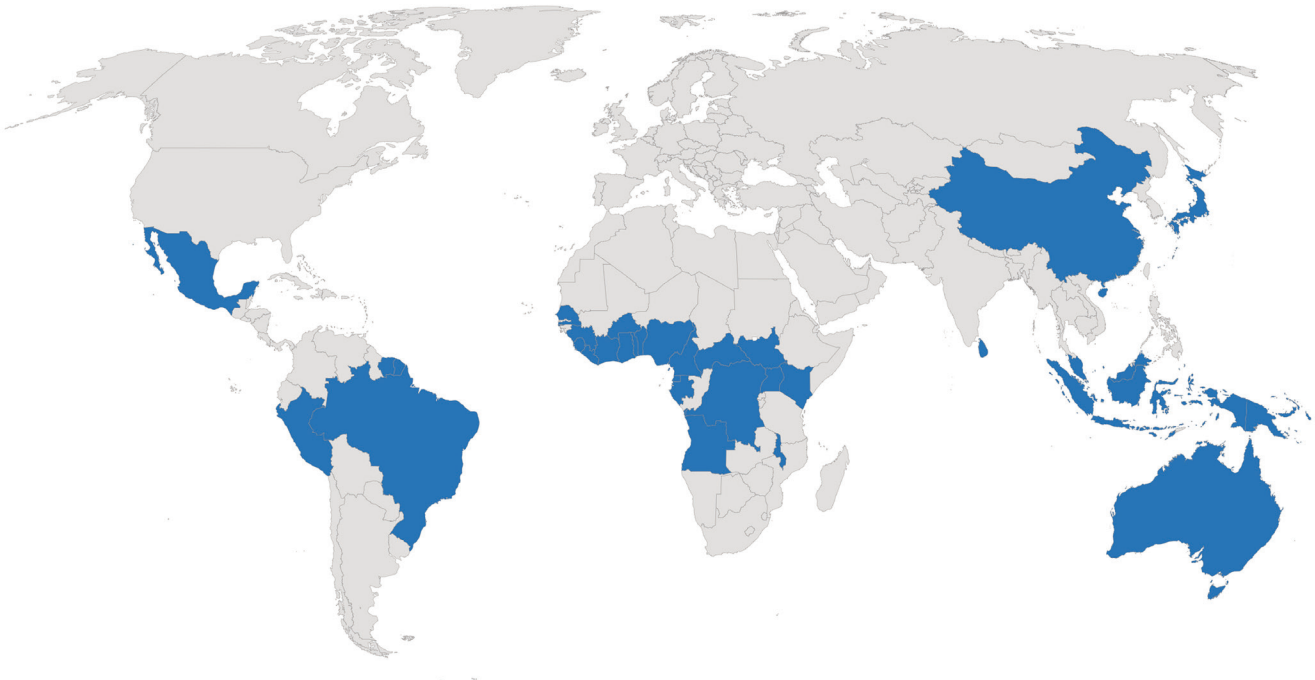

Figure 1. World map depicting countries with reported Buruli ulcer cases.

and rivers are more prone to infection (Brou et al. 2008; Einarsdottir and Huygen 2011). Changes in the ecosystem has been cited as a reason behind incidence of Buruli ulcer (Morris et al. 2014). Multiple lines of evidence have pointed out that amongst mycobacterial diseases, Buruli ulcer is the third common one after tuberculosis and leprosy (Van der Werf et al. 1999; Etuaful et al. 2005; Walsh et al. 2011). What starts as a painless nodule in the limbs, when left untreated progresses into severe ulceration and necrosis (Fig. 2) owing to the immunosuppression triggered by mycolactone (de Souza et al. 2012; Hall et al. 2014). Age, late diagnosis, and joint infections are some of the risk factors for Buruli ulcer severity (Tai et al. 2018). Immunization with BCG in a controlled setting offered small degree of protection (Smith et al. 1977). However, in the absence of suitable vaccine (Philips et al. 2014), surgery and combinatorial antibiotic treatment with rifampicin and streptomycin was recommended (Yotsu et al., 2015). In Japan, a regimen of rifampicin, levofloxacin and clarithromycin was followed (Sugawara et al. 2015). But numerous side-effects have been reported. Repurposed anti tuberculosis drugs proved to be ineffective against Buruli ulcer (Liu et al. 2018).

Explosion of genome projects in the last two decades, coupled with development of high-performance computational facilities and software have resulted in a wealth of information (Sur et al. 2010). This includes

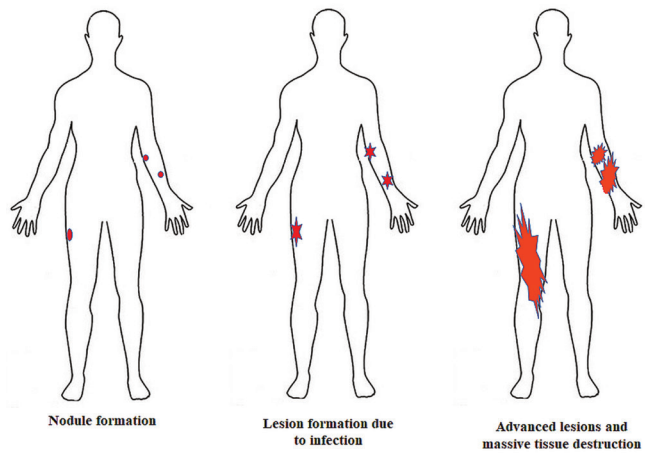

Figure 2. Buruli ulcer disease progression.

M. ulcerans as well. Here, we present a comprehensive review of the knowledge based on various bioinformatic analyses concerning *M. ulcerans* and Buruli ulcer. This is from the perspective of genomics and or comparative genomics, diversity, phylogenetic analyses, proteomics, vaccinomics, resistomics, etc. Additionally, we also comment on the opportunities of utilizing computational studies for controlling this pathogen.

Whole genome, comparative and population genomics research

Advances in molecular biology and genomics coupled with the publication of human genome in the early 21st century accelerated studies on whole genome. A necessity was felt to decode the huge deluge of information coming out from genome projects, aimed at enriching comparative genomics that could be applied to research on mycobacterial diseases (Zakham et al. 2011). While earlier genomic studies on identification of *M. ulcerans* concentrated on the use of 16SrRNA gene sequencing technology (Nakanaga et al. 2007), the whole genome sequencing of *M. ulcerans* Agy99 (Stinear et al. 2007) offered a new outlook into the biology of the pathogen. This was the first ever whole genome sequence of a *M. ulcerans* strain. It established the implication of reductive evolution and adaptation of the pathogen to specific niche (Stinear et al. 2007). Since 2007, several whole genome sequences of human pathogenic *M. ulcerans* strain Harvey, *M. ulcerans* subsp. *shinshuense* ATCC33728, *M. ulcerans* strain S4018, *M. ulcerans* strain CSURP7741, *M. ulcerans* strain SGL03, *M. ulcerans* strain P7741 etc. became available in the public domain. The genome sequence of *M. ulcerans* subsp. *shinshuense* ATCC33728 isolated from Japan and determined by 454 GS FLX Titanium technology, is made up of a 5.9 mb chromosome and a 166 kb plasmid (Yoshida et al. 2016). *M. ulcerans* strain S4018 isolated from a Buruli ulcer patient in Benin was sequenced on a MiSeq sequencer platform (Kambarev et al. 2017). *M. ulcerans* strain CSURP7741 isolated from French Guiana, was sequenced using a combinatorial approach of Nanopore and Illumina methods. It had some affinities with the frog pathogen *M. ulcerans* subsp. *lifandii* (Saad et al. 2019). Most of these genomes had a high GC content. *M. ulcerans* strain Harvey is the largest among human pathogenic *M. ulcerans,* having coding sequences more than 9000 coding sequences ((https://patricbrc.org/view/Taxonomy/2#view_tab=genomes&filter=and(keyword(Mycobacterium),keyword(ulcerans)). These genome sequences house valuable information that are crucial for understanding the phylogeny, pathophysiology, and lifestyle of this bacteria.

Over the years, comparative genomics research on different mycobacterial species have been the successful in providing valuable insights (Zakham et al. 2011). One such work focused on genes located within regions of difference accounting for 7% of *M. ulcerans* genome. The work demonstrated that gain of virulence plasmid along with deficiency of recognizable anti-virulence genes, catalyzed by IS2606 expansion. This armed the classical lineage with better capability with respect to virulence and transmissibility (Käser and Pluschke 2008). An extensive comparative genomics analyses from Benin using bioinformatic methods, resulted in the detection of 45 *M. ulcerans* specific proteins that could assist in the serodiagnosis of Buruli ulcer. This study underscored the importance of further research in generating antigenic repertoire of *M. ulcerans* (Pidot et al. 2010). That, *M. ulcerans* Agy99 showed affinity with *Mycobacterium leprae* Br4923, *Mycobacterium* sp. KMS and *Mycobacterium* sp. MCS was illustrated by a study with 14 mycobacterial genomes (Zakham et al. 2011). A comparison of whole genome sequences of thirty mycolactone producing mycobacteria and *M. marinum,* highlighted that *M. ulcerans* does not behave as a normal saprophyte. In all probability it had adapted itself to an aerobic and osmotically stable ecological niche that also protects it from light (Doig et al. 2012). Comparison of the *M. ulcerans* and *M. marinum* complex portrayed some fascinating aspects. The work highlighted mycolactone producing mycobacteria as a monophyletic group and stressed the importance to consider these bacteria as a single species i.e., *M. ulcerans* (Doig 2012). Moreover, the work also demonstrated the role of selective pressure and purifying selection in protein coding genes of *M. ulcerans.* Besides, the outcome also accentuated that speciation of *M. ulcerans* had no effect on codon translation (Doig 2012). Investigation of 21 mycobacterial genomes including *M. ulcerans* Agy99 divulged that although they differed in size, yet they had comparable high GC and low tRNA content. Besides, the ES1 locus extant in *Mycobacterium tuberculosis* and *M. marinum* was missing in *M. ulcerans* Agy99 (Zakham et al. 2012). A robust comparative genomics methodology was successful in detecting 424 essential genes from the genomes of *M. ulcerans* linked to carbohydrate and amino acid metabolism. Out of them, 236 were possible candidates for vaccine development. Furthermore, a number of enzymes associated with the cell wall, thiamine, histidine and protein biosynthesis pathways were also predicted to be prospective drug targets (Butt et al. 2012).

Increasing interest in mycobacterial comparative genomics prompted development of a user-friendly analysis platform named MycoCAP (Choo et al. 2015). This served as a suite for analyzing genomes from 55 *Mycobacterium* species including *M. ulcerans.* MycoCAP houses an assortment of web-based tools for searching, genome annotation, comparing genomes and virulence genes, determining phylogenetic relationships between mycobacterial strains and super classification. The developers of this trailblazing platform contemplate that, addition of newer genomes and their analysis will provide significant evolutionary insights (Choo et al. 2015).

The last decade also witnessed the use of whole genomes studies to figure out transmission and outbreak

of *M. ulcerans* in different countries. Research on whole genomes of *M. ulcerans* in clinical isolates from Ghana, Ivory Coast, Togo, Benin, and Nigeria, revealed widespread presence of the bacterium coupled with the existence of multiple genotypes in certain areas (Ablordey et al. 2015). The researchers opined that mobility of Buruli ulcer infected humans and livestock, from neighboring countries might have had contaminated the water bodies in different parts of Ghana. As a result, certain genotypes were introduced which later on transmitted among the population. This work stressed the need to undertake more whole genome surveys to understand the mechanism of genotype admixtures (Ablordey et al. 2015). Whole genome sequencing of isolates using NGS technologies from Benin and their comparison revealed incidence of exogenous Buruli ulcer reinfection (Eddyani et al. 2015). This incident demonstrated the importance of understanding transmission routes by targeted genome sequencing in Ouémé river valley. Another work based on whole genome amplification of crayfish samples in water bodies from Japan, illustrated seasonal emergence of *M. ulcerans* subsp. *shinshuense* (Luo et al. 2015). This study specified the link between contaminated water and incidence of Buruli ulcer.

Ever increasing number of mycobacterial genomes resulted in a plethora of information regarding cytochrome P450 monooxygenases (CYPs), a crucial enzyme for metabolic processes. Computational analysis of mycobacterial CYPs including those from *M. ulcerans* Agy99 demonstrated high diversity and their association with oxidation of steroids, fatty acids and terpenoids (Parvez et al. 2016). Human pathogenic mycobacteria have low count of CYPs which are crucial for lipid synthesis (Senate et al. 2019). Recently, a comparative study of CYPs from complete genomes of seven *M. ulcerans* strains provided insights into their lifestyle. Using CYPMiner software (Kweon et al. 2020) the study predicted 261 CYPs in the strains, classified into 35 and 38 families and subfamilies (Sur 2021). Although a few of them were diagnostic markers for some strains, there were 20 conserved families and subfamilies. While the flourishing family CYP140 was linked to mycolactone synthesis and pathogenesis, others were destined for lipid utilization. Interestingly, African strains showed similarities in their CYP profile (Sur 2021). Furthermore, the work revealed mutual association between CYP families and subfamilies.

Scientists have conducted population genomics studies applying bioinformatics methodologies, to ascertain the population structure and evolution of *M. ulcerans* in Africa. One such study using evolutionary trajectory and dynamics of *M. ulcerans* from Democratic Republic of Congo, Republic of Congo and Angola detected distinct sequence types. The research highlighted that a superior sublineage of *M. ulcerans* evolved in these countries and became endemic to hotspots in certain transmitted regions (Vandelannoote et al. 2019). This outcome once again pointed out the necessity for novel intervention-based health strategies, to disrupt transmission from localized outbreaks. Another study with whole genome sequences from clinical isolates in Melbourne, applying phylogeographic and Bayesian phylogenetic techniques divulged an interesting outcome. The investigation portrayed that *M. ulcerans* started migrating in eastern portion of Southeast Australia in the 1980's and gradually expanded to the western regions by increasing its population to a great extent (Buultjens et al. 2018). It once again emphasized the urgency to conduct environmental surveillance of pathogen mobility and have suitable interventional strategies in place to prevent migration and growth of endemic cases. It is clear from these sorts of research in Africa and Australia, that assessment of population structure and disease management based on comparative genomics data, is pivotal for controlling Buruli ulcer. In African countries and Australia with high burden of the disease, a localized assessment of *M. ulcerans* population using genomics (Vandelannoote et al. 2019a) can go a long way in treating Buruli ulcer.

Genomic diversity research based on computational techniques

Last decade of the 20th century witnessed a growing clamor for molecular typing of different strains of *M. ulcerans* from various parts of the globe (Jackson et al. 1995). Scientists were of the opinion that diversity studies would usher a new chapter for better understanding of Buruli ulcer epidemiology. Accessibility of genome sequence data of mycobacterial pathogens including *M. ulcerans,* signified the importance of studying genome polymorphisms to recognize pathogenic characteristics (Zhu et al. 2009). The necessity to have a database for mycobacterial genome polymorphisms gave birth to MyBASE. This user-friendly database accommodates information on genomic polymorphisms of different mycobacteria including *M. ulcerans*. It facilitates interpretation of diversity in pathogenicity, genome structure, evolution etc. (Zhu et al. 2009).

A single nucleotide polymorphism (SNP) profiling, using next-generation sequencing methodology from three strains of *M. ulcerans* demonstrated certain features (Qi et al. 2009). The Ghanaian isolate of *M. ulcerans* Agy99, when compared to a Japanese strain indicated 26564 SNPs in the latter. However, juxtaposition of *M. ulcerans* Agy99 with two other strains from Ghana revealed only 173 SNPs. This study illustrated that the Ghanaian clade

diverged from the Japanese strain 394-529 thousand years back, while two other Ghanaian subtypes diverged only 1000-3000 years back (Qi et al. 2009). One more study using high-throughput DNA sequencing data, of *M. ulcerans* genome isolates from Densu river basin of Ghana indicated sparse SNPs. Additional phylogenetic reconstruction analysis using SNP genotyping data divulged the ascendancy of a clonal complex and variants within it (Röltgen et al. 2010).

It was reported that multilocus sequence typing (MLST) based on housekeeping genes, of *M. ulcerans* isolates resulted in six contrasting genotypes from wide-ranging biogeographical regions of the world (Narh et al. 2014). Comparative DNA analysis between samples from water, soil, biofilms, and clinical samples, from Buruli ulcer patients in South Togo revealed similar *M. ulcerans* genetic profile (Maman et al. 2018). This study demonstrated riverine source of *M. ulcerans* infection in regions through which Haho and Zio rivers flow. MLST, short read DNA sequencing and SNP calling in a Japanese study, illustrated the difference between pigmented and non-pigmented colonies of *M. ulcerans* subsp. *shinshuense.* The pigmented and non-pigmented isolates differed only in 8 SNPs and 20 indels (Nakanaga et al. 2018). The latter was devoid of a large plasmid encoding regions for mycolatone biosynthesis, rendering it non-pathogenic in contrast to the former. Genome-wide association analysis of Buruli ulcer patients from Ouémé and plateau regions of Benin, revealed the role of lncRNAs and pathways linked to autophagy in the disease (Manry et al. 2019). These sort of genomic diversity studies using an assortment of computational techniques, enhanced our understanding of *M. ulcerans* regarding intraspecific divergence, geographical dissimilarities, virulence etc.

Research from Benin assessed bacterial diversity in skin lesions from individuals with Buruli ulcer. They performed a small-scale microbiome analysis using 16SrRNA sequencing, to gauge the composition of microbes from Buruli ulcer lesions, non Buruli ulcer lesions and skin samples of healthy persons (Leuvenhaege et al. 2017). The samples from Buruli ulcer lesions exhibited higher proportion of unassociated bacteria like *Bacteroides* and obligate anaerobes, in contrast to non Buruli ulcer lesions (Van Leuvenhaege et al. 2017). Since, skin microbiome is influenced by geography, genetics, climatic condition etc., its comparative study on a large scale should be used for estimating diversity in different populations (Nuhamunada et al. 2018). This work on Buruli ulcer lesions from Benin underlined that, additional microbiome-based analysis should be accompanied with standard microbiological studies.

In silico phylogenetics studies

Rapid improvement in "omics" data analysis and mycobacterial genomics research, has contributed to the understanding of evolutionary mechanisms (Bottai et al. 2014). One of the earliest works based on whole genome of *M. ulcerans* Agy99, indicated its evolution by lateral gene transfer and reductive evolution from *M. marinum* (Stinear et al. 2007). In fact, a number of factors viz., amassing 304 insertion sequence elements like IS2404 and IS2606, presence of 771 pseudogenes, genomic rearrangements, genome reduction, inability to tolerate sunlight, plasmid acquisition, mycolactone selective pressure and occurrence of foreign genes, contributed to its evolution and allowed it to colonize insects (Fig. 3a) by adapting to an arthropod ecosystem (Stinear et al. 2007). Moreover, the loss of ESX1 locus was also regarded as a survival strategy. Extensive comparative genomics analysis of insertions, deletions and genomic rearrangements from clinical isolates of *M. ulcerans* revealed that the microorganism evolved into five haplotypes (Käser et al. 2007). Two phylogeographically well-defined lineages were observed (Fig. 3a). The classical lineage underwent substantial genomic rearrangements and comprised of highly pathogenic genotypes concentrated mainly in Africa, Australia and Southeast Asia. They were over-represented by genes belonging to the PPE/PE families. On the other hand, the less pathogenic ancestral lineage housed environmental genotypes from China, South America and Mexico which showed similarity with *M. marinum* (Käser et al. 2007). There is strong evidence that, the classical and ancestral lineages diverged during the arrival of modern humans (Qi et al. 2009). However, the African isolates were not quite archaic and came into existence in the last 18,000 years (Stinear et al. 2000).

Some *in silico* analysis pointed out that, reductive evolution increased the pathogenic capacity of *M. ulcerans* by gaining a virulence plasmid pMUM001 (Demangel et al. 2009). Further comparative evolutionary genomic studies with two Ghanaian strains and one Japanese strain, demonstrated that the latter had an unstable genome compared to the former. Additionally, reductive evolutionary pressure was less among the Ghanaian strains (Qi et al. 2009). These were attributed to chromosomal rearrangements. Phylogenetic analysis of different mycobacterial species using 16SrRNA sequences, divulged the placement of *M. ulcerans* in a separate clade along with *M. marinum* (Zakham et al. 2012). A vast phylogenetic study with mycolactone producing mycobacteria including *M. ulcerans,* specified the role of pMUM plasmid housing genes for mycolactone biosynthesis to their advent (Doig et al. 2012). Add to this was the gain of cell wall associated genes and loss of cell wall antigens. This set

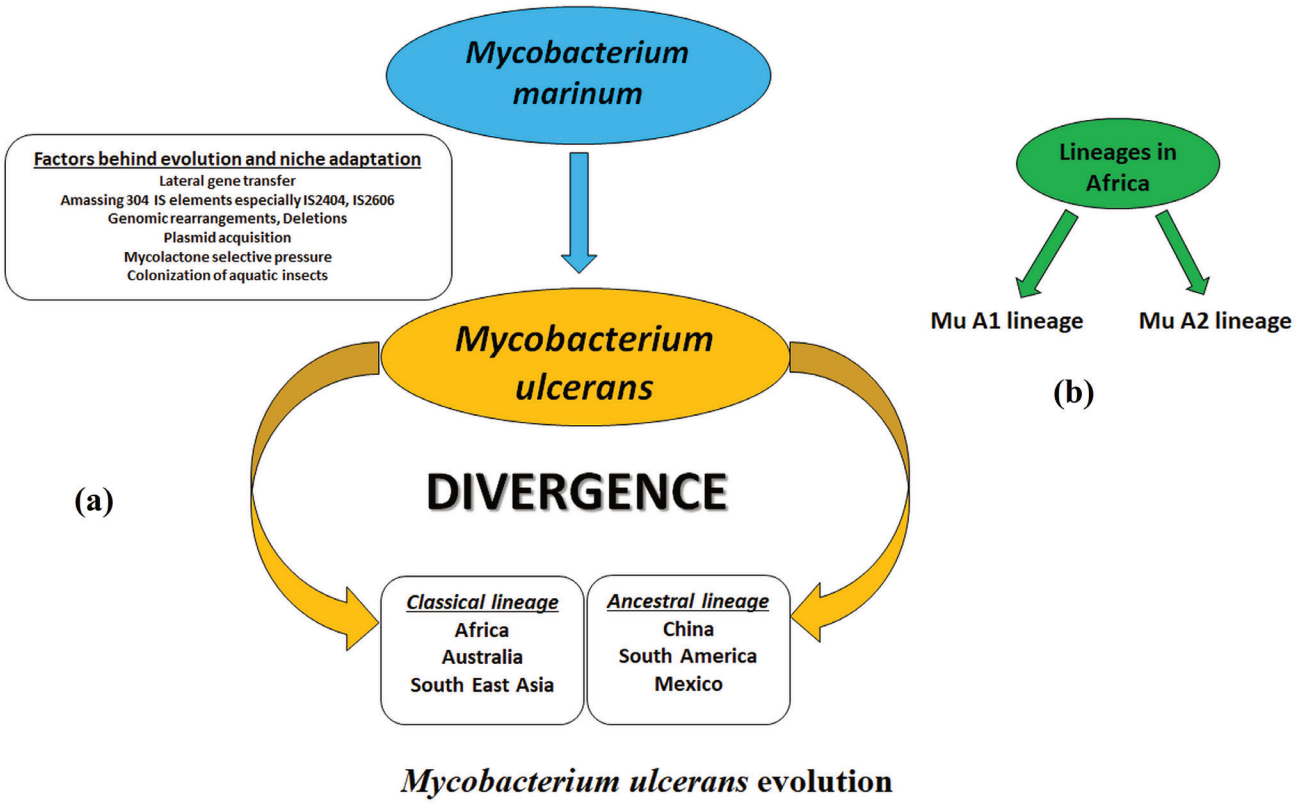

Figure 3. (a) Schematic depiction of the evolution of *Mycobacterium ulcerans* and its divergence into two major lineages (Stinear et al. 2007; Käser et al. 2007; Käser and Pluschke 2008). Note the factors responsible for evolution from *M. marinum* and niche adaptation. (b) Specific *M. ulcerans* lineages from Africa (Vandelannoote et al. 2017; Zingue et al. 2018).

off the process of cell wall remodeling that was crucial for the lifestyle of *M. ulcerans,* especially in its potential to form biofilms. Additional analysis of the mycolactone producing mycobacterial complex showed that, *M. ulcerans* were in all probability transferred between Africa and Australia not long ago. This was ascribed to genetic drift and deletion of some genes, linked to metabolism and respiration that were futile for adaptation to distinct ecological niches (Doig et al. 2012).

Phylogenetic analysis based on complete mycobacterial genomes, hypothesized occurrence of shared common mobile elements between *M. ulcerans* and *M. marinum* (Reva et al. 2015). Moreover, reticulate network analysis also supported the close relationship between these bacteria. Evolutionary reconstruction studies and Bayesian analysis of *M. ulcerans* from 11 endemic regions of Africa, identified two specific *M. ulcerans* lineages (Fig. 3b) housed in the continent (Vandelannoote et al. 2017). While the Mu_A1 lineage was from 68 BC, the Mu_A2 lineage was introduced by humans in 1800 AD (Zingue et al. 2018). The close relation of the latter with isolates from Papua New Guinea was attributed to anthropogenic activities.

One recent phylogenetic study with *M. ulcerans* from French Guiana and its comparison with global strains, utilizing core and accessory genomes threw up interesting facts. Five distinct lineages were identified by maximum likelihood phylogeny (Reynoud et al. 2019). Out of these, the L1.2 lineage was completely independent. The French Guinean strains were clustered together (Reynoud et al. 2019). Research using complex whole genome sequences of *M. ulcerans* from Australian counties, underscored the impact of microevolution. It was found that three *M. ulcerans* complex clones, were the reason behind uptick of cases in Southern Australian counties compared to non-endemic counties in rest of Australia (Saad et al. 2020).

The outcome of *M. ulcerans* phylogenetic studies accentuated the interplay of myriad factors including phylogeography and human activities, which were responsible for variation amongst lineages, pathogenic lifestyle, and adaptation to specific niches.

Proteomics of *M. ulcerans* and Buruli ulcer

Prokaryote research has reaped the benefit of rapid advances in the field of proteomics (Burley and Bonnano 2002). One of the pioneering computational proteome-based studies concerning *M. ulcerans,* was a comparative analysis of *Mycobacterium tuberculosis* strains and NTMs that included *M. ulcerans* Agy99 (Zakham et al. 2012). It used a BLAST matrix to perform genomic analysis of the predicted proteomes (Zakham et al. 2012). The work revealed low similarity between *M. tuberculosis* strains, *M. ulcerans* Agy99 and MAV complex. A classic investigation involving quantitative proteomics and transcript level analysis, highlighted the implication of culture conditions on the regulation of mycolactone toxin in *M. ulcerans* (Deshayes et al. 2013). Data from 2D gel electrophoresis and mass spectrometry analysis demonstrated that during pathogenesis and lesion formation, mycolactone altered the cytoskeleton and hindered collagen biosynthesis (Gama et al. 2014). It reiterated that mycolactone toxin was associated with reduction in collagen content in Buruli ulcer lesions. Another worker (Sarpong 2018) used high throughput mass spectrometry data to explore differentially expressed proteins from fast and slow healing Buruli ulcers. Highly expressed proteins viz., IFI30, PSME3, CD74, C4A linked to MHC class I, MHC class II and complement pathways showed better promise in healing Buruli ulcer (Sarpong 2018).

Vaccinomics research

The genome sequencing of a number of *M. ulcerans* strains opened up numerous possibilities for identifying potential vaccine candidates. Currently, there is no vaccine preventing Buruli ulcer (Mangas et al. 2020). A limited transient protection lasting for a year or so has been reported in individuals administered with *Mycobacterium bovis* BCG vaccine (Einarsdottir and Huygen 2011). Thus, development of a suitable vaccine is important for preventing *M. ulcerans* infections and Buruli ulcer severity. In the post genomic era, a study on mice revealed the efficacy of priming species specific Ag85A-DNA and homologous protein boosting, in eliciting strong Th1 immune responses against *M. ulcerans* infection (Tanghe et al. 2008). Efforts to develop inactivated vaccines, DNA/protein vaccines by targeting the mycolactone toxin, enzymes synthesizing mycolactones, mycobacterial proteins and *M. ulcerans* specific proteins demonstrated some degree of humoral and cellular responses (Huygen et al. 2009; Pidot et al. 2010; Einarsdottir and Huygen 2011). Comparative genomics methods were used for serological assessment of the antigens (Pidot et al. 2010). However, none of them were tested in clinical trials. The last decade saw increased interest in *in silico* identification of potential antigens and peptides for designing new vaccine candidates. Development of mycobacrvR package using reverse vaccinology and integrative immunoinformatic approach, served as a means for designing epitope-based vaccine candidates against mycobacterial diseases (Chaudhuri et al. 2014). This package used an assortment of 20 algorithms for determining adhesins with extracellular and surface localized characteristics (Chaudhuri et al. 2014). Analysis of the whole proteome of *M. ulcerans* Agy99 identified 36 adhesin and adhesin like proteins. Out of these, 26 potential vaccine candidates were identified by the enhanced reverse vaccinology method (Chaudhuri et al. 2014). Others attempted to develop a recombinant vaccine from *Mycobacterium marinum* against Buruli ulcer (Hart et al. 2016).

Mycobacterial secretory proteins have been the target of vaccine researchers since they are known to induce immune responses (Gcebe et al. 2016). There is evidence that the PE/PPE family of genes possess the capability to elicit Th1 response against tuberculosis (Bennan et al. 2017). Some workers studied the highly antigenic PE-PGRS family proteins from the whole proteome of *M. ulcerans* Agy99, to predict multi-epitope vaccine against it (Nain et al. 2020). They applied a robust integrated vaccinomics methodology (Fig. 4) by utilizing a wide array of algorithms. Initially, they selected 15 suitable epitopes which interacted with HLA binding alleles and demonstrated significant population coverage on a global coverage. This was followed by designing the vaccine chimera. The designed construct revealed antigenic, immunogenic, and non-allergenic characteristics. Docking and MD simulation studies demonstrated binding affinity with TLR2 receptor (Nain et al. 2020). Furthermore, *in silico* cloning, codon optimization and *in silico* immune response simulation analysis postulated that the vaccine construct is a suitable candidate for generating immune response against *M. ulcerans* Agy99 (Nain et al. 2020). Although, experimental studies are necessary to substantiate the findings.

In silico studies on antibiotic resistance and exploration of natural products as anti Buruli ulcer drugs

The World Health Organization (WHO) has approved the use of antibiotics for primary treatment of Buruli ulcer lesions (Omansen et al. 2019). Although there are some uncertainties surrounding antibiotic therapy, further treatments using surgery, physical therapy, analgesia, and community therapy are followed in many countries

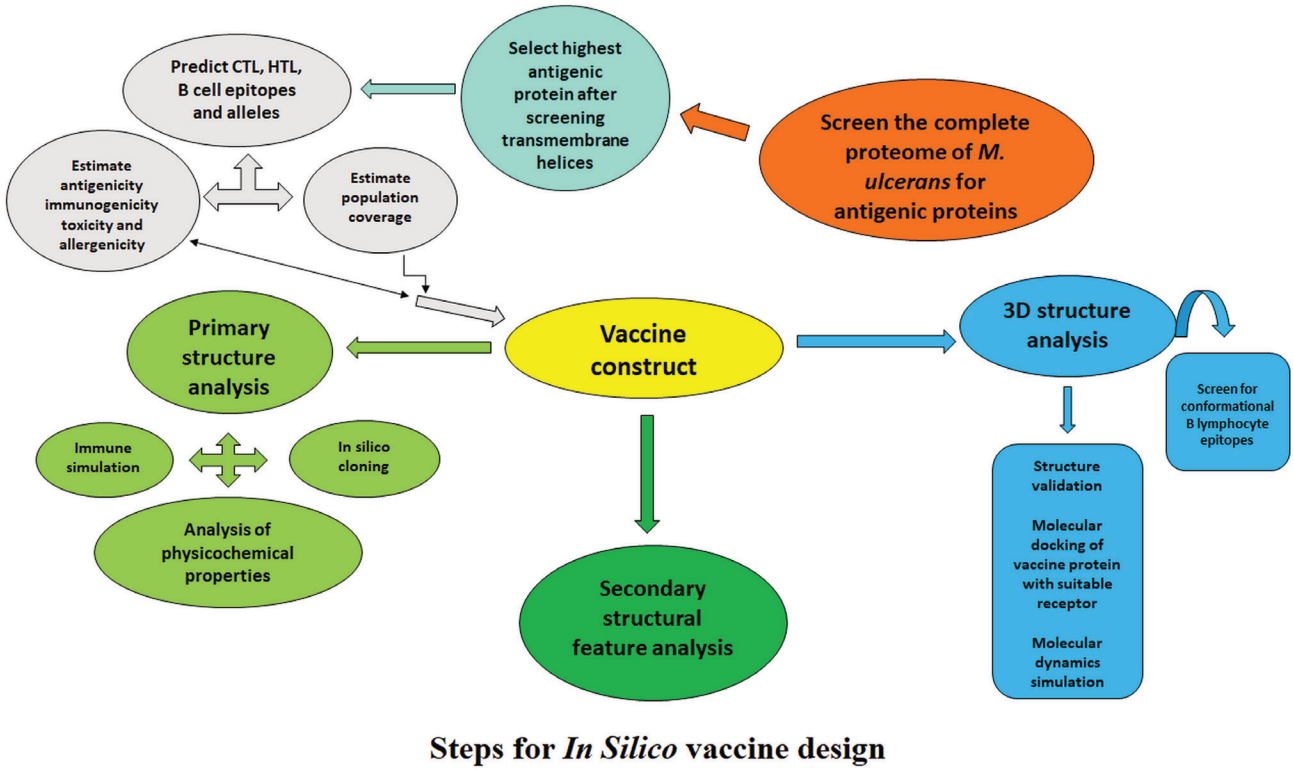

Figure 4. Schematic representation of the *in silico* vaccine design methodology (Nain et al. 2020). CTL = cytotoxic T-lymphocyte, HTL = helper T-lymphocyte.

(Omansen et al. 2019). In recent years, development of a number of bioinformatics tools and databases, opened up the possibility of exploring microbial whole genome sequencing data for investigating antibiotic resistance (Hendriksen et al. 2019). An *in silico* study highlighted the occurrence of 14 antibiotic resistance genes in *M. ulcerans* Agy99. Single mutation in *katG* and *pncA* genes were responsible for resistance to isoniazid and pyrazinamide drugs (Gupta et al. 2017). This rendered them useless against *M. ulcerans* Agy99. Additionally, this study predicted that, using a cocktail of antibiotics like rifampin, streptomycin, azithromycin, clarithromycin etc., probably assisted in overcoming the impact of mutation and may control Buruli ulcer (Gupta et al. 2017). Nonetheless, these sorts of studies should be expanded to other *M. ulcerans* strains as well.

The growing concern of antibiotic resistance, coupled with the lack of potent antibiotics against late stage Buruli ulcer has troubled the clinicians. Despite this, identification of suitable lead compounds that could act as anti Buruli ulcer drug in an *in silico* study from Ghana (Kwofie et al. 2018) showed promise. They screened isocitrate lyase from *M. ulcerans and* generated its three-dimensional structure using molecular modeling. After refinement, molecular dynamics simulation, and active site prediction, the structure was used for molecular docking with AutoDock (Kwofie et al. 2018). Virtual screening of the AfroDb for natural compounds, followed by docking resulted in 20 compounds showing reasonable affinity for isocitrate lyase. Further physicochemical analysis and ADMET testing narrowed upon ZINC38143792, ZINC95485880, and ZINC95486305 as best leads that could be suitable for experimental validation (Kwofie et al. 2018). These lead compounds known to possess inhibitory (Mujovo et al. 2008) and anti-bacterial properties (Kwofie et al. 2018) possibly restricted disease progression by neutralising isocitrate lyase.

Conclusion and future perspectives

Buruli ulcer caused by *M. ulcerans* is a neglected tropical disease. *M. ulcerans* has been understudied in comparison to other mycobacterial pathogens. The major burden of Buruli ulcer is often borne by the poor. This has resulted in socioeconomic problems. In the recent years, a deluge of information from *M. ulcerans* genome projects, coupled with state-of-the art research in comparative genomics,

population genomics, pathogen mobility/transmissibility, pathogen phylogeny, proteomics and designing vaccine candidates using *in silico* tools, has provided fantastic insights. This has revolutionized our understanding of Buruli ulcer. Phylogenetic and population genomics studies have illustrated the significance of incorporating microbial phylogeographic data in the analysis, since it highlighted information about strain origin, spread of the bacterium and migration of hosts. This has met with success in some regions of Africa and Australia wherein, early detection of active cases, surveillance and treatment resulted in reduced transmission and disease incidence (Vandelannoote et al. 2017, 2019). However, lot more needs to be done. Governments and international organizations should understand the necessity to finance Buruli ulcer research so as to improve disease control measures and minimize the burden. There is a need to have an international network of researchers with diverse expertise to foster technological innovations. This should be aimed at expanding high precision cost effective sequencing methodologies, advancing development of new tools/software, large scale targeted studies on genome variability/diversity in countries/regions with high prevalence, investigating the pattern of host microbiomes and identification of new vaccine candidates. Information from genomes could also be used to develop potent diagnostic and treatment regimens. Moreover, genome-based investigations should be increasingly conducted to understand insect vector-based transmission of Buruli ulcer. This is important since, *M. ulcerans* has crafted an ecological niche for itself in aquatic insects. Furthermore, there is a need to broaden newer genotyping strategies and boosting genomic diversity studies. Sequencing more genomes from different locations could aid such studies. Additionally, *in silico* techniques should be applied to understand the nature of pMUM plasmid and mechanism of mycolactone toxin immunosuppression (Einarsdottir and Huygen 2011). The information from such studies can aid in the development of effective vaccines and therapeutic drugs against *M. ulcerans,* using immunoinformatics and immunogenomics approaches. Pharmaceutical companies and biomedical industries should take initiatives to validate the vaccine constructs designed by robust *in silico* techniques. Computational studies on antimicrobial inhibitors and drug compounds from indigenous plants should be encouraged. This aspect of research is lacking. Clinicians and pharmaceutical scientists should assist bioinformaticians in this regard to combat the pathogen. The bottom line is to have a multidisciplinary effort in place, to better understand the epidemiological and transmission factors of this challenging and steadily evolving bacterium.

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