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MYCOBACTERIAL TYPE VII SECRETION SYSTEM: A POSSIBLE NOVEL TARGET FOR ANTI-TUBERCULOSIS DRUGS

Kamali-Kakhki, R.; Aryan, E.*

Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Both authors contributed equally to this work

*ehsanaryan@hotmail.com

Abstract

Several secretion systems have been identified and extensively studied in Gram-negative bacteria. Some of these systems such as type III secretion system (T3SS) directly inject bacterial effector molecules into the host cells. Therefore, they are among the most critical bacterial virulence factors and play a major role in bacterial pathogenesis. It has been proposed and investigated to use the bacterial secretory pathways as an unusual target for development of new antibacterial drugs. In this context, type VII secretion system (T7SS) has recently been identified and widely studied in *Mycobacterium tuberculosis*. Understanding about structural and effector components, mechanisms of action and diversity of T7SS would possibly open the doors towards developing novel anti-tuberculosis therapeutics, vaccines and/or diagnostics targeting a new site in the bacterial cell. Future researches will determine the feasibility of this idea.

Key words: Type VII secretion system, mycobacteria, anti-tuberculosis drugs

Introduction

Antibiotic resistance is a major and emerging problem in the treatment of bacterial infections with a lethal effect on some diseases such as tuberculosis [1]. Since antibiotics mainly target a unique enzyme or process in the bacterial cells such as protein or DNA biosynthesis, mutations can easily be generated and highly be selected resulting in rapid resistance to antibiotics even after several years of their clinical use. This has led to less interest for investment of resources for research and development of antibiotics targeting new sites in bacterial cells. As phage therapy of bacterial infections has been noted previously [2], there is an urgent need to find alternative approaches for treatment of bacterial infections that slow the development of resistance to antibacterial agents.

Agents that target virulence factors can inactivate bacterial pathogens, allowing the host immune responses to eradicate the pathogen [3]. Therefore, virulence-targeted drugs are the most promising options to fight against infections.

It would seem that bacteria develop resistance mechanisms to virulence-targeted drugs more slowly than antibiotics, because these drugs would have little effect, if any, on the critical functions of the cell like DNA replication or protein biosynthesis [4]. In other words, virulence traits are not usually essential for growth and viability of a bacterial cell, and therefore environmental factors causing selection of resistant cells would not have significant effect. On the other hand, virulence-targeted drugs are only effective on bacteria that contain the target, and in contrast to antibiotics, they would not have deadly effect on the bacteria that constitute the host indigenous microbiota which are beneficial to the host.

Virulence-targeted drugs could not only be a precious substitute for antibiotics, but also they can be administered in combination with antibiotics to increase therapeutic effect of the antibiotic and lower the chance of resistance development.

There are diverse virulence mechanisms in bacterial pathogens that can be potential targets for antibacterial drug development, such as mechanisms that bacteria use for host cells attachment and invasion (i.e., adhesions), bacterial toxins and their secretion systems, etc.

Secretion of effector proteins by pathogenic bacteria facilitates their persistence and colonization in the human host [5], making the bacterial secretion systems an important virulence factor. Toxic proteins are translocated across the bacterial envelope by these secretion systems or directly injected into the cytoplasm of host cell where they exert their toxic effect on the cell.

Generally, there are at least six different secretion systems in Gram-negative bacteria, namely types I to VI secretion systems. In Gram-positive bacteria, secretion of proteins mainly occurs via general secretory (Sec) or twin-arginine protein translocation (Tat) pathways [6]. The unusual cell envelope of mycobacteria consists of an inner cytoplasmic membrane, an arabinogalactan layer, a peptidoglycan layer, and a unique outer membrane known as mycomembrane. The latter mainly contains long-chains of mycolic acids substituted with free glycolipids [7], which are not exist in most bacteria. Although mycobacteria have been considered as Gram-positive bacteria, because of their uncommon structure and highly complex cell envelope, they use various secretion pathways such as the SecA1 secretory pathway [8], SecA2 secretory pathway [9], Tat pathway [10], and a recently identified secretion system known as type VII secretion system (T7SS) or ESX (early secretory antigenic target [ESAT]-6 system) [11]. T7SS has widely been studied in *Mycobacterium tuberculosis*; however it is not exclusive to mycobacteria. Similar secretion systems have been found in other bacterial orders and families including *Pseudonocardiales, Micromonosporales, Streptomycetales, Catenulisporales, Frankiales, Actinomycetales, Gordoniaceae, Micrococcales, Corynebacteriaceae, Propionibacteriales, Tsukamurellaceae, Glycomycetales, Nocardiaceae, Streptosporangiales, Bifidobacteriales, Coriobacteriales, Segniliparaceae* [11, 12]. T7SS is one of the most essential mechanisms involved in host–pathogen interactions and virulence of *M. tuberculosis* and other pathogenic mycobacteria. It therefore would be an ideal candidate for diagnosis of tuberculosis (TB) by newer molecular methods such as loop-mediated isothermal amplification [13, 14], or a promising target for development of new anti-tuberculosis drugs [3, 15, 16]. The main goal of this review is to describe the structure, components, and diversities of mycobacterial T7SS, providing necessary substrates for future research and development of new antimicrobials, vaccines or diagnostics.

The history of identification of T7SS in mycobacteria

T7SS was identified with the discovery of 6-kDa early secreted antigenic target (ESAT-6 or EsxA) protein in the culture filtrate of *M. tuberculosis* [17]. Studies on differences between the genome of virulent *M. tuberculosis* and avirulent *M. bovis* BCG specified deletion of a genomic region designated as RD1

(region of difference 1), carrying nine genes encoding ESX-1 substrates and its structural components, from the chromosome of currently used live attenuated anti-tuberculosis vaccine (i.e., BCG) [18, 19].

This genomic region has been also deleted from the chromosome of *M. microti* [20], which has been used as a live attenuated vaccine in the 1960s. Interestingly, complementation tests showed that secretory properties could be resumed only by integration of the entire RD1 region of *M. tuberculosis* into the genomes of *M. bovis* BCG and *M. microti* [21]. This finding confirmed that the RD1 locus encodes secretion system of mycobacteria and enhances the bacterial virulence. Since there is no similarity between this new secretion system and other recognized secretion systems, it is also referred to as type VII secretion system (T7SS).

Type VII secretion system (T7SS)

T7SS was first described in *M. tuberculosis* H37Rv and its related gene clusters were later named ESX (ESAT-6 Secretion System) [22]. The genome of *M. tuberculosis* contains five distinct ESX gene clusters, each of which encodes a defined T7SS, termed ESX-1 to ESX-5 [23]. In addition to the five chromosomal ESX loci, a plasmid-encoded ESX system named ESX-P has recently been reported [24]. This secretion system involves in survival and virulence of *M. tuberculosis*, and contributes to the secretion of certain proteins involved in the bacterial pathogenesis across the mycobacterial complex cell envelope [25]. The ESX secretion system is composed of several T7SS-type-specific proteins (EspA, EspB, EspC, EspG, etc), T7SS-conserved proteins (EccB, EccC, EccD, MycP, etc), and secreted proteins like CFP-10, ESAT-6, and PPE [26].

ESX-3 is the only T7SS that is required for iron transport into the bacterial cells and it therefore is necessary for the viability of *M. tuberculosis* [27], whereas ESX-1 and ESX-5 are involved in the pathogenesis of *M. tuberculosis* and other pathogenic mycobacteria [28]. It has been shown that ESX-1 and ESX- 5 secrete various effector proteins affecting host cell functions, although some aspects about their substrates, molecular targets and mechanisms of secretion are yet to be recognized [29].

ESX-P (plasmid-encoded ESX) has a major role in diversification of ESX, exchange of related genes between the plasmid and bacterial chromosome, evolution of mycobacterial pathogenesis and ESX-associated virulence factors of *M. tuberculosis*, making its adaptation with new hosts and environments [24]. In this way, T7SS is involved in interstrain genetic exchange and host-pathogen interactions. Therefore, its better understanding might help to recognize some new ways for diagnosis [13, 14], prevention [30, 31] and treatment [3, 15, 16] of tuberculosis and other pathogenic mycobacteria in the future.

ESX-1

ESX-1 is the most studied T7SS, because it has a crucial role in the virulence of pathogenic mycobacteria through the secretion of effector molecules like EsxA (ESAT-6), EsxB (CFP-10) and other associated proteins [32].

Besides the members of *M. tuberculosis* complex, ESX-1 secretion system has been also identified in a number of other pathogenic mycobacterial species, including *M. kansasii* type I [33] and *M. leprae* [34], *M. bovis* [35], *M. marinum* [36, 37], *M. smegmatis* [38] and *M. vanbaalenii* [39]. However, lack of this secretion system in the genome of mycobacterial opportunistic pathogens, like *M. avium*, *M. ulcerans* and *M. paratuberculosis* indicated that these mycobacteria migt have an alternative infection cycle independent of ESX-1 (see Table 1) [40].

It is clear that ESX-1 has an important role in bacterial pathogenesis, because deletion of RD1 from chromosome of *M. tuberculosis* reduces the bacterial virulence, as its re-entering into the genome of *M. bovis* BCG will cause enhanced virulence [41].

Recent researches have shown that the EsxA·EsxB complex is a major virulence factor and it may have an important effect on the host-pathogen interactions. Biologically, EsxA is more active than its natural partner in the complex (EsxB). EsxB, as a part of the complex, is a chaperone protein to deliver EsxA to the target site for its action [42].

Several key features in behavior of mycobacterial cells are influenced by the presence or absence of ESX-1 during the infection. Although, comparison at the genome level of *M. bovis* BCG and *M. tuberculosis* indicated 99.9% homology betrween them [43], however, the absence of ESX-1 in *M. bovis* BCG results in a number of differences in its pathogenesis with *M.tuberculosis* in terms of promoting host cell necrosis and cell-to-cell spread [44], impairment or induction of autophagy [45] and apoptosis [46], stimulation of conjugation [35], inflammasome activation [47], $CD8^+$ T-cell responses [48], prevention of Toll-like receptor (TLR) signaling [49] and induction of type I interferon and interleukin-8 (IL-8) production [50]. All these features seem to be related to deletion of RD1 BCG.

ESX-1 have been extensively studied and many

studies have confirmed that ESX-1 system is required in the early stages of infection of macrophages through stimulating intracellular growth of pathogenic mycobacteria [32].

However, some questions have still remained to be answered. For example, it has been shown that ESX-1 is involved in preventing the phagosomal maturation in *M. marinum* and *M. tuberculosis* [51], while some studies have indicated the role of ESX-1 in membrane lysis of phagolysosomes and releasing of the mycoacteria into the host cell cytosol [52]. Therefore, EsxA has been reported as a pore-forming cytotoxin [53].

There are some inconsistency in these findings. Despite the fact that EsxA is secreted by non-pathogenic *M. smegmatis,* this bacterium is not able to translocate across the membrane of phagosomes into the host cytoplasm [40]. There are three possiple explanations for these conflicting data: i) EsxA sequence diversity between *M. smegmatis* and *M. tuberculosis*for may results in its different function in these bacteria; ii) due to inability of non-pathogenic *M. smegmatis* for intracellular replication, certain cellular functions such as cell division may possibly be needed for secretion of EsxA and phagolysosomal membrane lysis; iii) in pathogenic mycobacteria, EsxA might help in the secretion of another effector molecule which is needed for translocation. It should be considered that these studies were conducted by EsxA alone without its partner EsxB [40].

Although the active role of ESX-1 in the virulence of pathogenic mycobacteria has been completely proved, it has an absolutely different role in environmental mycobacteria such as *M. smegmatis*. It involves in the process of conjugation and is essential for conjugal DNA transfer by the donor cell [35]. It seems that these very different functions of ESX-1 could be due to substrate variation.

ESX-2

ESX-2 locus is located exactly adjacent to the ESX-1 region, but different ESX systems may be present among the species containing T7SS. For example, *M. marinum*, a fish mycobacterial pathogen which has a high homology in its ESX-1 system with *M. tuberculosis,* does not encode ESX-2 sysytem (see Table 1) [54].

Generally, ESX-2 cluster is present in *M. tuberculosis* [55], *M. bovis* BCG [56], *M. bovis* [56], *M. avium* [57] and *M. paratuberculosis* [26]. However, certain mycobacterial species such as *M. smegmatis* [23], *M. vanbaalenii* [39], *M. leprae* [34], *M. kansasii* type I [33], *M. marinum* [58] and *M. ulcerans* [59] do not carry this gene cluster (Table 1).

Although the function of ESX-2 system in *M. tuberculosis* remains unknown, mutagenesis studies in *M. tuberculosis* have shown that mutants for all ESX-2 genes could not survive [60]. Unfortunately, only limited predictions of its putative functions are available.

ESX-3

It should be considered that ESX-3 system is one of the most conserved systems in the genome of mycbacterial species, probably because it plays an important role in iron/zinc homeostasis [61]. ESX-3 is present in all available mycobacterial genomes such as *M. tuberculosis* [27], *M. Bovi* BCG [27], *M. bovis* [27], *M. avium* [57], *M. paratuberculosis* [62], *M. kansasii* type I [33], *M. marinum* [63], *M. ulcerans* [63], *M. smegmatis* [23], *M. vanbaalenii* [39] and *M. leprae* [34] (Table 1).

Mycobacteria acquire iron by at least two classes of siderophores as the iron scavenging systems: mycobactin and exochelin [64]. Although mycobactin class of siderophores exists in approximately all mycobacterial species [64], however, exochelin is only produced by fast-growing mycobacteria like *M. smegmatis* [64]. One study has suggested that mycobactin is required for uptake of iron by ESX-3 system [27]. Therefore, ESX-3 gene cluster is not essential for *M. smegmatis* unless exochelin biosynthesis and uptake pathways are inactive [27, 60]. Therefore, it is clear why mutation in ESX-3 genes is not lethal for *M. smegmatis*, because it produces exochelin as an alternative siderophore. However, the function of Esx-3 in iron acquisition via the mycobactin pathway is not comparable with *M. leprae*, which has ESX-3 but is not able to produce mycobactin [65]. Therefore, ESX-3 might be involved in an alternative mycobacterial iron acquisition pathway. Some studies have shown that mycobacterial Esx-3 is required for bacterial growth during the infection of macrophages [66], because they are not able to acquire metal ions by the use of mycobactin in macrophages. It has been reported that ESX-3-deficient *M. bovis* BCG significantly grows slowly in macrophages [66]. Therefore, Esx-3 is essential for both in vivo and in vitro growth of *M. bovis* BCG, unlike ESX-1 which is essential for in vivo growth of pathogenic mycobacteria, indicating dissimilar requirement of mycobacteria to different types of T7SS.

Like ESX-1, ESX-3 also secretes paralogous small proteins. As Esx-1 encodes EsxB (CFP-10) and EsxA (ESAT-6), Esx-3 encodes EsxG and EsxH proteins [17, 38, 41]. Several reports indicate that secretion of

EsxG and EsxH is related to Esx-3 function. Mutagenesis studies show that these proteins are required for mycobactin-mediated iron acquisition [65].

In *M. tuberculosis*, esx-3 genes are regulated by high concentrations of zinc or iron through Zur and IdeR transcription regulators, respectively. It therefore is suggested that ESX-3 involves in both zinc and iron deficiency of *M. tuberculosis*, however in *M. smegmatis*, regulation of esx-3 genes is only conduted by iron through IdeR [61], indicating that it only involves in iron deficiency in this mycobacterial species. The exact role of ESX-3 and its secreted effectors, EsxG·EsxH complex, in zinc and iron acquisition is unknown yet, although it has been noted that ESX-3 could play a role in iron acquisition through the mycobactin pathway [27], and the specific Zn^{2+}-binding site present on the EsxG·EsxH complex may indicate its important role in zinc ion acquisition [63].

ESX-4

Comparative genomic analysis has shown that ESX-4 gene cluster is the most ancestral ESX system, and also probably the smallest *esx* locus in the genome of mycobacterium. Because it harbors smaller number of genes than other *esx* loci [23, 26, 55]. This hypothesis is based on this observation that the last mycobacterial ancestor has already preserved an ESX-4 system and the only *esx* gene clusters found in other GC-rich Gram-positive bacteria are *esx*-4-like loci [26, 40].

Other mycobacterial ESX clusters may have evolved through gene diversification events, duplication and insertion of additional genes from ESX-4-like systems. For example, the insertion of *pe* and *ppe* genes into the *esx*-4 locus might have occurred during the bacterial evolution [23]. Because all *esx* gene clusters, apart from *esx*-4, encode at least one or more members of *pe-ppe* gene pairs that are named according to their characteristic N-terminal proline-glutamic acid (PE) and proline-proline-glutamic acid (PPE) motifs [23].

Although many PE-PPE proteins are not restricted to T7SS and can be encoded out of the *esx* locus [39], however all tested PE-PPE proteins are secreted by ESX systems [67].

Functional information about ESX-4 is limited, however genetic screening of *M. tuberculosis* have shown that *esx*-4 genes are not crucial for bacterial survival [60]. According to a published study, this T7SS is not involved in the early stages of mycobacterial infection, although the important role of ESX-4 in mycobacterial persistent infection remains to be identified [40].

The *esx*-4 gene cluster is present in *M. tuberculosis* [40], *M. bovis* BCG, *M. bovis* [68], *M. avium* [57], *M. paratuberculosis* [39], *M. kansasii* type I [33], *M. marinum* [69], *M. ulcerans* [59], *M. smegmatis* [23, 69] and *M. vanbaalenii* [39]. *M. leprae* does not contain an endogenous copy of *esx*-4 [34] (Table 1).

ESX-5

ESX-5 has been studied in more details in *M. tuberculosis* and *M. marinum* [67, 70], and is only present in slow-growing mycobacteria. It appears to be highly conserved in these species [23]. Several studies about ESX-5 deficiency in *M. tuberculosis* and *M. marinum* have shown that this system involves in the secretion of PE and PPE proteins which contain polymorphic GC-rich sequences (PGRS) [22, 28, 58, 70]. ESX-5 system is essential for full virulence of *M. tuberculosis* in vitro. It has been also demonstrated that growth of mycobacterial strains is affected by mutatioans in *esx*-5 genes, resulting in the virulence-attenuated strains [58]. As mentioned earlier, ESX-5 is strongly responsible for secretion of PE and PPE proteins in *M. tuberculosis* and *M. marinum* [58, 67, 71]. These proteins are localized in mycobacterial cell surface and are thought to be involved in bacterial antigenic variation, because of the highly polymorphic nature of their C-terminal domain [58, 71]. According to these reports ESX-5 has an important role in bacterial virulence and evasion of the host immune responses [72].

There are plenty of *pe* and *ppe* genes in the genome of *M. tuberculosis*, which constitute about 7.1 % of its genomic capacity [73]. A number of these proteins are encoded out of the *esx* loci. Another study has reported that *esx*-5 mutants are unable to transport both ESX-5-encoded and many non-ESX-5-encoded PE and PPE proteins across the mycobacterial membrane. Since these proteins need to be secreted out of the mycobacterial cell envelope, secretion of some PE and PPE proteins suggests the presence of an intact *esx*-5 loci in the genome of *M. tuberculosis* [67]. ESX-5 system is present in the genome of various slow-growing mycobacteria such as *M. tuberculosis* [58], *M. bovis* BCG [67], *M. bovis* [67], *M. avium* [57], *M. paratuberculosis* [57, 74], *M. kansasii* type I [33], *M. marinum* [58] and *M. ulcerans* [58], however several non-pathogenic mycobacterial species including *M. vanbaalenii* [39] and *M. smegmatis* lack this system (Table 1) [23].

M. leprae is an exception. Although *M. leprae* contains a functional ESX-5 system, however many of its *esx*-5 genes exist as pseudogenes [23, 34].

ESX-5 is involved in late stages of macrophage infection. ESX-5-deficient pathogenic mycobacteria are unable to modulate cytokine responses of macrophages [28, 67]. One study has reported that ESX-5 mycobacterial secreted proteins are associated with increased production of pro-inflammatory cytokines during granuloma formation, induction of host cell death and releasing of IL-1β [28].

It has been approved by strong evidences that ESX-5 function is also necessary for IL-1β release upon infection with *M. marinum* [28]. The mechanism of regulation, however, remains to be clarified. In contrast to ESX-1 [53], mutation in *esx*-5 genes does not affect bacterial translocation into the cytosol of host cells in *M. tuberculosis* and *M. marinum* [28, 29].

ESX-P

Plasmid-encoded ESX system, ESX-P, has recently been reported [24]. There is also extensive diversity in plasmid-encoded ESX clusters, in addition to five chromosomal-encoded ESX in the genus of mycobacterium. To date, at least seven new ESX profiles have been recognized (ESX-P clusters 1 to 7). Of 14 plasmids, ranging from 97 kb to 615 kb in size, which contain ESX motifs, ESX-P clusters 1 to 3 are carried by multiple plasmids, whereas the remaining four ESX-P (ESX-P clusters 4 to 7) are only found in one plasmid (Table 2).

Existence of different ESX systems have suggested that plasmid-encoded ESX clusters were significantly involved in ESX diversification and genetic exchanges of ESX associated genes and systems. Analysis of phylogenetic tree of ESX sequences has shown that different types of ESX-P were grouped with certain families of chromosomal ESX. For example, ESX-P cluster 4 is grouped together with the family of ESX-3. In most cases, the gene order within different systems of ESX-P is also consistent with chromosomal-encoded ESX system leading to recombination hypothesis between the chromosomal and plasmid-encoded ESX system. The data set of a study and analysis of results reject this hypothesis and no recombination events have been detected. In general, these data show that ESX-P systems constitute authentic functional and diversified plasmid-specific ESX families [24].

Conclusion

Pathogenic mycobacteria use different strategies to survive and persist in eukaryotic cells. The ability of *M. tuberculosis* in stimulating host cell death is one of the examples. It is clear now that type VII secretion system plays an important role in the secretion of effector proteins during mycobacterial pathogenesis. The components of T7SS are also considered as virulence factors and therefore potential vulnerable targets in designing new anti-tuberculosis agents for pathogenic mycobacteria [75, 76]. In recent years, research on T7SS and its role in mycobacterial pathogenesis have become an interesting research topic. Although there are currently limited information about ESX-2 and ESX-4, however more data are available for ESX-1, ESX-3 and ESX-5 systems, which have been considered essential for viability and pathogenicity of the bacterium.

Despite definite involvement of ESX-1 system in the pathogenesis and granuloma formation of mycobacterial disease, its effector molecules and their role have not been completely explained. This secretion system is required in early stages of the infection helping translocation of mycobacteria into the host cell cytosol. The potential function of ESX-3 in zinc and iron homeostasis elucidates the role of this secretion system in viability of mycobacteria. Unlike ESX-1, ESX-5 is not involved in bactarial translocation into the host cell cytosol, however it seems to play an important role in the late stages of macrophage infection and affects host–pathogen interactions by modulation of macrophage cytokine responses. In spite of these differences, both secretion systems (ESX-1 and ESX-5) induce macrophage cell death. Accordingly, T7SS plays major roles in different stages of mycobacterial infection. More research on this secretion system may help to recognize some ways for development of new diagnostics, prevention and treatment of tuberculosis and other pathogenic mycobacteria. In this regard, several studies have reported potential vaccines targeting other virulence mechanisms of *M. tuberculosis* to prevent the disease [30, 31].

This review allows to recognize the extensive diversity of ESX systems (chromosomal and/or plasmid-encoded ESX) in mycobacteria and their important role in mycobacterial pathogenesis. Future studies are required to define the exact role of T7SS effector molecules in mycobacterial pathogenesis with the hope of developing new anti-tuberculosis drugs that target the vital components of this secretion system.

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Table 1. The presence or absence of ESX clusters in mycobacterial species.

	ESX-1	ESX-2	ESX-3	ESX-4	ESX-5
M. tuberculosis	+	+	+	+	+
*M. kansasii**	+	+	+	+	+
M. marinum	+	-	+	+	+
M. smegmatis	+	-	+	+	-
M. vanbaalenii	+	-	+	+	-
M. avium	-	+	+	+	+
M. ulcerans	-	-	+	+	+
M. paratuberculosis	-	+	+	+	+
M. leprae	+	-	+	-	?
M. bovis	+	+	+	+	+
BCG	-	+	+	+	+

+, Presence of ESX clusters; -, Absence of ESX clusters; ?, Present but many of its genes are pseudogenes;
*, ESX clusters 2 to 5 are absent from *M. kansasii* type I.

Table 2. Characteristics of the identified mycobacterial ESX-plasmids.

Strain	Plasmid name	Size (kb)	Reference
M. abscessus subsp. bolletii strain 5625	pMBOL	97	[24]
M. abscessus subsp. bolletii strain 50594	plasmid 2	97	[77]
M. kansasii ATCC 12478	pMK12478	145	[78]
M. marinum E11	pRAW	114	[79]
M. yongonense 05-1390	pMyong1	123	[80]
M. chubuense NBB4	pMYCCH.01	615	Lucas, S. et al. 2012*
M. chubuense NBB4	pMYCCH.02	144	Lucas, S. et al. 2012*
M. gilvum PYR-GCK	pMFLV01	321	Copeland, A. et al. 2007*
M. smegmatis	pMYCSM01	394	Lucas S. et al. 2011*
M. smegmatis	pMYCSM02	199	Lucas S. et al. 2011*
M. smegmatis	pMYCSM03	164	Lucas S. et al. 2011*
M. sp. KMS	pMKMS01	302	Copeland, A. et al. 2006*
M. sp. KMS	pMKMS02	217	Copeland, A. et al. 2006*
M. sp. MCS	plasmid 1	215	Copeland, A. et al. 2006*

* According to Dumas E., et al. (2016) publicly released genome sequence.