Discovery of novel Mycobacterial lasso peptide biosynthetic gene clusters and structure prediction of the resulting peptide

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Abstract: Peptidic natural metabolits potentially represent a rich source of medically relevant compounds. Lasso peptides are one example of this group in which there is a great interest in the last 10 years due to their unique structure. They are produced by bacteria and are defined by the presence of a knotted structure known as “lasso fold” [1]. Apart from the exclusive structure, a wide range of biological activities are known for these compounds, including antimicrobial, enzyme inhibitory, and receptor antagonistic activities. Novel lasso peptides are being constantly discovered and analyzed by different techniques together with their structural identification which provides information for elucidating the mechanisms of its activity and basis for modifications [2]. Therefore, in the present study, we have identified biosynthetic gene clusters (BGCs) of lasso peptides among the underestimated Mycobacterial strains whose complete genome were available using genome mining techniques. The prepetide sequence was then translated based on the highest score open reading frame (ORF) predicted in genome mining step. The leader peptide cleavage sites were predicted using the comparative analysis with known lasso peptides to yield the core peptides for structure prediction step. The structure of unique potent lasso peptides were then predicted via RiPPMiner which is a machine learning based tool for deciphering chemical structures of bioactive ribosomal peptides [3]. Accordingly, of 137 mycobacterial genomes mined for lasso peptides only 3 showed to possess the BGCs within which 2 were unique. The two lasso peptides named LP1 and LP2 found in *Mycobacterium neoaurum* VKM Ac-1815D and *Mycobacterium sinense*, respectively with 9 and 16 aminoacids for their core peptide. LP1 shows similar structure to bacterial head-to-tail-cyclized peptdies while LP2 were more likely to be categorized as propeptin-like lasso peptide. Moreover, Clustal Omega-generated phylogenetic tree based on the sequence of prepeptide showed that the mycobacterial peptides are evolutionary more similar to *Asticcacaulis excentricus* and *Sphingopyxis alaskensis* thus the peptides are more similar to proteobacteria rather than actinobacteria. According to structure, sequence and evolutionary comparative analysis, the two novel lasso peptides are potent to be antibacterial. This can be true since it is reported that lasso peptides can inhibit the growth of mycobacteria which suggests the role of producing these peptides by mycobacteria for competing with their relatives. Conclusively, our study first proves the potential of mycobacteria as the most underestimated actinobacterial members in terms of secondary metabolite production, and moreover, the approach can be implemented as a first line of bioactive peptide discovery which greatly decrease time and costs. Further studies and verifications on the best candidates elucidated in *in silico* procedure can reveal the most promising peptides.

Keywords: Lasso peptides; Genome mining; Mycobacteria; Structure prediction; Antibacterial peptides