

Abstract

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) is a technique developed in the late 1990s enabling the two-dimensional mapping of a broad variety of biomolecules present at the surface of a sample. In many applications including pharmaceutical studies or biomarker discovery, the distribution of proteins, lipids or drugs, and metabolites may be visualized within tissue sections. More recently, MALDI MSI has become increasingly applied in microbiology where the versatility of the technique is perfectly suited to monitor the metabolic dynamics of bacterial colonies. The work described here is focused on the application of MALDI MSI to map secondary metabolites produced by *Bacilli*, especially lipopeptides, produced by bacterial cells during their interaction with their environment (bacteria, fungi, plant roots, etc.). This chapter addresses the advantages and challenges that the implementation of MALDI MSI to microbiological samples entails, including detailed protocols on sample preparation (from both microbiologist and mass spectrometrists points of view), matrix deposition, and data acquisition and interpretation. Lipopeptide images recorded from confrontation plates are also presented.