Matrix Assisted Laser Desorption and Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has gained more and more popularity for the identification of bacteria. Identification of anaerobic bacteria can be cumbersome and time-consuming. MALDI-TOF MS gives the opportunity to obtain a reliable identification in a few minutes. Several studies have been performed in which MALDI-TOF MS has been used for the identification of anaerobic bacteria e.g. Prevotella sp., Fusobacterium sp., Clostridium sp., Bacteroides sp. and gram-positive anaerobic cocci (GPAC). In each of these studies MALDI-TOF MS was shown to be superior over conventional identification methods. The importance of an extensive database containing the reference spectra was also shown in these studies. Several of these studies first compiled a database which was subsequently used to identify unknown strains. Strains not identified by MALDI-TOF MS were mostly species of which no reference spectra were present in the database. In a multicenter study two commercially available MALDI-TOF MS systems, Bruker MS and Shimadzu MS, were compared in their ability to identify anaerobic bacteria. The Shimadzu MS system performed better than the Bruker MS system. However, an update of the Bruker MS database resulted in an improved performance, comparable to that of the Shimadzu MS system. This confirms the importance of an extensive database. At this moment a correct species identification is obtained, when compared to the 16S rRNA gene sequencing, for roughly 60 % of the anaerobic bacteria encountered in human clinical specimens. More strains can be identified when the available databases are optimized for the identification of anaerobic bacteria.