

Characterization of bacteria using mass spectrometric detection of polymerase chain reaction products

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The polymerase chain reaction (PCR) is a very useful technique for producing many copies of (*i.e.*, amplifying) a desired region of DNA from any given organism. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a promising alternative to conventional electrophoresis and hybridization methods for detecting the products of PCR amplification. MALDI-MS offers advantages such as speed and accuracy of *m/z* measurement over existing techniques, and we are working to overcome remaining limitations that presently prevent its widespread use. PCR requires a mixture of salts, buffers, enzymes, and other reagents, while MALDI-MS requires fairly pure solutions of DNA for sensitive and accurate detection, so an intermediate purification step is necessary. To this end, a rapid reverse-phase purification technique can be implemented using a 96-well microtiter plate format. Another problem in MALDI-MS of DNA is the inhomogeneous nature of the dried mixture of matrix and PCR product, which necessitates laborious manual positioning of the desorption laser to various locations around the dried sample to obtain abundant signal. To eliminate this searching, we are studying polymeric substrates that can yield a more homogeneous dried sample spot from which abundant signal can be obtained independent of desorption laser position on the spot. Finally, to produce DNA in the size range amenable to MALDI-MS detection, PCR assays can be redesigned to yield smaller products (<100 bases) that retain equivalent information to conventional larger products. These strategies are illustrated in the development of MALDI-MS methods for detecting the *pmoA* gene in methanotrophic bacteria that can co-metabolize chlorinated pollutants, and are thus of interest for bioremediation at sites of industrial contamination.

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