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Cloning, expression, and immunological characterization of the P30 protein of Mycoplasma pneumoniae.

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Source

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Abstract

Mycoplasma pneumoniae, a self-replicating cell wall-deficient prokaryote, has a differentiated terminal organelle that is essential for cytadherence and gliding motility. P30, an important protein associated with the terminal organelle, is required for the cytadherence and virulence of M. pneumoniae. P30 is a transmembrane protein with an intracytoplasmic N terminus and an exposed C terminus. In the present study, we amplified and sequenced the full-length p30 gene of Mycoplasma pneumoniae directly from 18 Indian asthmatic patients. Sequence diversity was observed in the p30 genes from 16 clinical samples when the sequences were compared with the sequence of strain M-129. We also successfully expressed a fragment of the p30 gene (P30B) that includes the complete C-terminal proline-rich amino acid sequences in different Escherichia coli expression systems. The maltose binding protein (MBP)-P30B fusion protein was recognized by M. pneumoniae-infected patient sera in immunoblots, and the protein was immunogenic in mice. We further analyzed the reactivity of the MBP-P30B fusion protein with patient sera in an enzyme-linked immunosorbent assay (ELISA) and compared it with the reactivity obtained with a commercial kit (the Serion ELISA Classic kit). The sensitivity and the specificity of the in-house ELISA were 78.57% and 89.47%, respectively. This study suggests that the P30 protein can be used as an antigen along with other adhesin proteins for the immunodiagnosis of M. pneumoniae infection.

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