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Expression and proteolytic cleavage of Lav, a putative virulence autotransporter of nontypeable Haemophilus influenzae

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Haemophilus influenzae, a gram negative coccobacillus, is part of the normal flora of the human upper respiratory tract. Unencapsulated (nontypeable) strains of H. influenzae (NTHi) cause pneumonia, sinusitis, and otitis media, and complicate chronic lung disease. Strain R2866 is unusually invasive and was isolated from a child with meningitis. R2866 and many other NTHi have a gene termed lav, whose encoded protein belongs to the family of virulence-associated autotransporters. A nearly identical gene is found in the meningococcus, which obtained it by horizontal transmission from H. influenzae. Lav is a phase-variable outer membrane protein, with the ON or OFF phase controlled by the number of tetranucleotide repeats downstream of the initiating codon. We are investigating the function and localization of Lav. Most H. influenzae autotransporters are adhesins. Lav is not a primary adhesin, but preliminary experiments suggest it may improve adherence to human lung tissue cells. Autotransporters consist of three primary domains, an N-terminal signal peptide, a C-terminal beta-barrel domain that forms a pore in the outer membrane, and a middle "passenger" domain that is the effector part of the protein and is exported through the pore. In most autotransporters, the "passenger" domain is proteolytically cleaved from the beta-domain, usually remaining bound at the cell surface. We are testing to see whether Lav is similarly cleaved, by comparing whole cell extracts, outer membrane fractions, and culture supernatants. Cell fractions are Western-blotted and probed with antibodies specific for the passenger domain or the C-terminus. Strains include R2866 with Lav in the ON and OFF phase, a phase-locked ON derivative of R2866, a null Lav mutant, and E coli and H. influenzae Rd engineered to express Lav. A 20-kDa fragment with the passenger N-terminal epitope was found in culture supernatants of strains expressing Lav but not in those of control non-expressing strains.