***Pseudomonas aeruginosa in vivo* Outer Membrane Proteome: Role of Simple Porins**

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Pseudomonas aeruginosa is a major opportunistic pathogen. Because of its high intrinsic resistance to antibiotics and the emergence of additional resistance mechanisms, it has become one of the most dangerous infectious agents in hospital-acquired infections. It is often assumed that the *P. aeruginosa* outer membrane simple porins crucially determine antibiotic translocation and efficacy. Indeed, inactivation of one of its 35 different simple porins, OprD, decreases *P. aeruginosa* susceptibility specifically to carbapenems. However, if similar links also exist between other antibiotics and simple porins remains largely unclear. To determine which porins *P. aeruginosa* expresses, we have developed a sensitive targeted mass spectrometry-based proteomic approach. The results showed that diverse *P. aeruginosa* strains including human clinical isolates, express only a rather small subset of simple porins under various *in vitro* conditions. As expected, OprF was the dominant simple porin. In addition, five other simple porins (OprD, FadL, OprG, OprQ, OprE) were commonly detected. Six other porins were detected at low and variable levels (Tsx, OpdQ, OpdC, OpdH, OpdP, OprB). All other porins were below our detection threshold of ca. 30 molecules per cell. To assess the functional relevance of these porins, we generated a series of single or multiple gene deletions on PA14 strain. Antimicrobial susceptibility testing revealed that none of the simple porins other than OprD affected minimal inhibitory concentrations (MIC) of diverse antibiotics. Surprisingly, even a delta34 strain lacking all simple porins had identical MIC values compared to parental PA14 (except for carbapenems where the delta34 strain phenocopied the *oprD* mutant). This strain grew normally on rich media but had severe growth defects on minimal media containing single carbon sources. Interestingly, we did not detect any compensatory expression of cryptic porins in various multiple knock-outs. These results suggest a role of simple porins in nutrient uptake, but do not support current models of antibiotics membrane translocation. In addition of simple porins, alternative translocation routes likely exist. These findings have major implications for the mechanism of antibiotic action against *P. aeruginosa* and developments of urgently needed novel drugs.