Pseudomonas aeruginosa is an opportunistic pathogen responsible for a number of different human infections and is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. P. aeruginosa infections are difficult to treat due to a number of antibiotic resistance mechanisms and the organisms propensity to form multicellular biofilms. Epidemic strains of P. aeruginosa often dominate within the lungs of individual CF patients, but how they achieve this is poorly understood. One of the ways strains of P. aeruginosa can compete, is by producing chromosomally encoded bacteriocins, called pyocins. Three major classes of pyocin have been identified in P. aeruginosa: soluble pyocins (S-types) and tailocins (R- and F-types). In this study, we investigated the distribution of S- and R-type pyocins in 24 clinical strains isolated from individual CF patients and then focused on understanding their roles on inter-strain competition. We found that (i) each strain produced only one R-pyocin type, but the number of S-pyocins varied between strains; (ii) R-pyocins were crucial for strain dominance during competition assays in both planktonic cultures and within biofilms; (iii) purified R-pyocins demonstrated significant antimicrobial activity against established biofilms. Our work provides clear support for a key role played by R-pyocins in the competition between P. aeruginosa strains, and may help explain why certain strains and lineages of P. aeruginosa dominate and displace others during CF lung infection. Furthermore, we demonstrate the potential of exploiting R-pyocins for therapeutic gains in an era when antibiotic resistance is a global concern.