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Iron- and 4-hydroxy-2-alkylquinoline-containing periplasmic inclusion bodies of *Pseudomonas aeruginosa*: A chemical analysis

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Abstract

Dark aggregated particles were seen on pellets of iron-rich, mid-logarithmic phase *Pseudomonas aeruginosa*. Transmission electron microscopy of these cells showed inclusion bodies in periplasmic vacuoles. Aggregated particles isolated from the spent medium of these cells contained iron as indicated by atomic absorption spectroscopy and by electron paramagnetic resonance spectroscopy that revealed Fe^{3+}. Scanning electron microscopy/energy dispersive X-ray analysis of whole cells revealed the presence of iron-containing particles beneath the surface of the cell, indicating that the isolated aggregates were the intracellular inclusion bodies. Collectively, mass spectroscopy and nuclear magnetic resonance spectroscopy of the isolated inclusion bodies revealed the presence of 3,4-dihydroxy-2-heptylquinoline which is the *Pseudomonas* quinolone signaling compound (PQS) and an iron chelator; 4-hydroxy-2-heptylquinoline (pseudan VII), which is an iron chelator, antibacterial compound and precursor of PQS; 4-hydroxy-2-nonylquinoline (pseudan IX) which is an iron chelator and antibacterial compound; 4-hydroxy-2-methylquinoline (pseudan I), and 4-hydroxy-2-nonylquinoline N-oxide.

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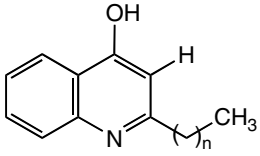
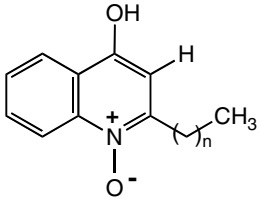
1. Introduction

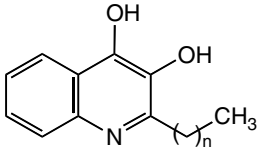
The Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic pathogen that is capable of causing a variety of diseases in the immunocompromised host as well as chronic lung disease in the cystic fibrosis patient. The organism is known for secreting numerous virulence factors into its environment, as well as compounds collectively called 4-hydroxy-2-alkylquinolines (HAQs, Fig. 1). Two HAQs produced by *P. aeruginosa* are 4-hydroxy-2-heptylquinoline (pseudan VII) and 4-hydroxy-2-nonylquinoline



n = 0 4-hydroxy-2-methylquinoline (pseudan I)
n = 6 4-hydroxy-2-heptylquinoline (pseudan VII)
n = 8 4-hydroxy-2-nonylquinoline (pseudan IX)



n = 6 4-hydroxy-2-heptylquinoline-N-oxide
n = 8 4-hydroxy-2-nonylquinoline-N-oxide



n = 6 3,4-dihydroxy-2-heptylquinoline
n = 8 3,4-dihydroxy-2-nonylquinoline

Fig. 1. Chemical structures of relevant HAQs.