Abstract

A different class of quorum sensing (QS) signal molecules, known as 2-alkyl-4-quinolones (AQs) have been discovered in *Pseudomonas aeruginosa.* The Pseudomonas Quinolone Signal (PQS) is arguably the most important QS signal in this signalling cascade, playing a vital role in regulating a myriad of virulence factors. A novel chemically defined medium with PQS as the sole carbon and energy source was developed and used in the successful enrichment and isolation of a PQS-degrading bacterium from rain forest soil samples. Based on 16S rDNA sequence analysis, the bacterium was classified as a strain of *Achromobacter xylosoxidans*. The PQS degradation activity was assessed via TLC analysis coupled with *Pseudomonas aeruginosa* PAO1Δ*pqsA* CTX-*lux*::*pqsA* bioreporter overlay, which induces bioluminescence in response to PQS. In the presence of succinate as the co-substrate, *A. xylosoxidans* was capable of rapidly degrading 20 μM PQS with the subsequent accumulation of a fluorescing compound that does not induce the bioreporter. Liquid chromatography – mass spectrometry (LCMS) and Fourier transform mass spectrometry (FTMS) analyses of the PQS degradation compound indicated that *A. xylosoxidans* degraded PQS via a hydroxylation reaction. To our knowledge, this is the first report of the discovery of a PQS degrading bacterium. In exploring PQS signaling inhibition, a PQS QS inhibitor selector system (PQSIS) was constructed. A *P. aeruginosa pqsA* mutant (PAO1 Δ*pqsA*) strain lacking the *pyrF* gene (encoding orotidine-5'-phosphate decarboxylase) was constructed. A plasmid-borne copy of the gene fused to a *pqsA* promoter forms the selection system. This construct relies on the induction of cell death due to fluoroorotic acid (FOA) sensitivity when grown in a minimal medium containing FOA and PQS. The presence of a PQS QS inhibitor will prevent cell death. PQSIS-3 can potentially be applied in the screening of novel PQS inhibitors among natural and synthetic compound libraries.

Abstrak

Molekul pengesanan kuorum yang dikenali sebagai 2-alkyl-4-quinolones (AQs) merupakan kelas baru yang telah ditemui di *Pseudomonas aeruginosa*. Dalam kelas ini, Pseudomonas Quinolone Signal (PQS) adalah jenis molekul pengesanan yang paling penting dan memainkan peranan utama dalam regulasi factor-faktor virulen. Suatu media minimal baru yang telah ditambahkan PQS sebagai sumber karbon dan nutrien telah diciptakan. Dengan menggunakan media ini, kami berjaya memperolehi bacteria yang mampu menguraikan PQS daripada sumber tanah. Melalui penjujukan gen 16S rDNA, bakteria ini ditentukan sebagai *Achromobacter xylosoxidans*. Aktiviti penguraian PQS dikaji menerusi analisis TLC serta penglapisan bio-pengesan *Pseudomonas aeruginosa* PAO1Δ*pqsA* CTX-*lux*::*pqsA*. Apabila ditambahkan suksinat sebagai co-substrat, *A. xylosoxidans* mampu mengurai 20 μM PQS dengan akumulasi sejenis sebatian yang berpendar. Analisis-analisis dengan menggunakan 'Liquid chromatography-mass spectrometry (LCMS) serta 'Fourier transform mass spectrometry (FTMS)' menunjukkan bahawa PQS telah diuraikan melalui proses hydoksilasi. Ini merupakan laporan pertama mengenai pencarian bacteria pengurai PQS. Dalam mengkaji inhibisi pengesanan PQS, satu sistem pemilihan penginhibisi pengesanan kuorum untuk PQS telah diciptakan. Mutan *pqsA* dalam *P. aeruginosa* yang kekurangan gen *pyrF* (untuk orotidine-5'-phosphate decarboxylase) telah dibuat. Plasmid yang mempunyai salinan gen ini telah difiuskan dengan promotor pqsA untuk menyediakan system pemilihan. Penghasilan ini sensitive terhadap fluoroorotic acid (FOA) akan mati jika dikulturkan dalam media yang mengandungi FOA dan PQS. Kehadiran penginhibisi pengesanan kuorum PQS akan mencegah kematian cell dan system ini berpotensi untuk diaplikasikan dalam pencarian penginhibisi PQS baru di kalangan sebatian alam semulajadi dan juga sebatian sintetik.

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