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Detection of Diverse *N*-Acyl Homoserine Lactone Signalling Molecules Among Bacteria Associated with Rice Rhizosphere

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Abstract

Bacterial communities communicate, regulate and coordinate their cooperative activities and physiological process by releasing, sensing and responding to small diffusible signal molecules such as acyl homoserine lactones (AHLs), auto-inducing oligo-peptides (AIPs) and autoinducer-2, a process referred to as Quorum sensing (QS). The QS mediated communication in rhizosphere associated bacterial communities significantly influence traits governing plant–microbe interactions. This study aimed to identify AHL-mediated QS signals in bacterial communities associated with rice rhizosphere using two AHL biosensors reporter strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NTL4 (pZLR4). Approximately 375 bacterial isolates isolated from rice rhizosphere and screened using both the biosensors, detected 49 (13%) AHL positive isolates. The BOX-Polymerase Chain reaction (BOX-PCR) fingerprinting profiles of the 49 AHL positive isolates represented 11 distinct cluster groups. Subsequent 16S rRNA gene sequence analysis identified 11 different species affiliated to two different phyla; predominantly γ-proteobacteria, representing 5 genera and 1 genus in α-proteobacteria. Thin-layer chromatography (TLC) analysis detected diverse AHL profiles among the 11 AHL positive isolates with both substituted and unsubstituted acyl side chains of C4, C6 and C8 carbon. Further, AHL production in *Acinetobacter lactucae, Aeromonas popoffii, Serratia oryzae,* and *Rhizobium wuzhouense* is being reported for the first time. Detection of diverse AHLs from different groups of rhizobacteria associated with rice indicates that these signalling molecules may be involved in the regulation of rhizobacterial behaviour and symbiotic plant–microbe interactions. Future research on the role of AHLs in trans-kingdom communication particularly plant–microbe interaction using synthetic microbial community will enable in evaluating and developing potential plant specific bioproducts.

Introduction

Rhizobacterial communities are an integral part of the plant ecosystem which significantly influences the plant growth and alleviates abiotic and biotic stresses [1]. Plant-associated bacterial communities are reported to produce diverse signalling molecules which are involved in inter, intra and cross-kingdom communication [2–5]. The AHL producing

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bacterial communities are highly diverse and fall within a large number of species among α-, β- and γ- proteobacteria [6, 7] and are represented by 10–20% of the cultivable bacteria associated with the rhizospheric regions [8]. A diverse group of plant-associated Gram-negative proteobacteria isolated from the rhizosphere, use QS regulatory system to synthesize autoinducer signalling compounds of *N*-acyl homoserine lactones (AHL) to synchronize their cell density, molecular and metabolic pathways [6–9]. These signalling molecules are involved in the manifestation of numerous plant-associated phenotypes such as biofilm formation, colonization of plant root systems [7, 10, 11], induction of systemic resistance (ISR) in plants [12, 13], regulation of antibiotic and antifungal compound production [14, 15], regulation of plant gene expression and physiological features by altering the level of phytohormone production [16, 17]. The presence of diverse AHL-QS signalling molecules has been reported in more than 100 species of proteobacteria including *Pseudomonas, Agrobacterium, Rhizobium,*