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UHPLC-MS/MS, an Alternative Solution to Conventional Biosensor Approach for Quorum Sensing Signaling Molecules Detection in Complex Environmental Samples

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Introduction

Quorum sensing (QS) signaling is critical for coordinating the social behaviors of bacteria, i.e., regulating biofilm development[1]. Acylhomoserine lactones (AHLs) consist of a homoserine lactone ring and an acyl side chain, at variable lengths, oxidation states and saturation levels, are the most common signals employed by the bacteria. As AHLs are highly susceptible to elevated temperatures, alkaline conditions, and are often degraded rapidly by other microbes living within the same niche, it is always a real challenge to characterize and quantify AHLs in the natural environments[2]. Conventionally, AHLs are detected by biosensor-dependent assays, which are time-consuming and with limited sensitivity detecting all range of AHLs[3]. Here, a UHPLC-MS/MS method is developed to overcome the limitations of the biosensor-based AHL detection.

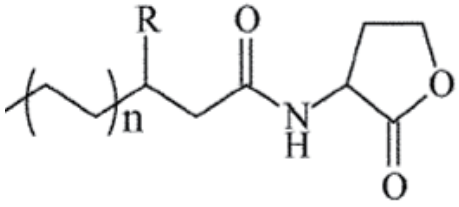

Fig. 1 General chemical structure of AHLs where R represents functional groups (H, O or OH) and n represents 1-15 carbons.

Materials and Methods

Naturally occurring AHLs, from the activated sludge of a lab-scale bioreactor system, were extracted by dichloromethane. The samples extracted were analysed on UHPLC-MS/MS tandem quadrupole system (Shimadzu LCMS-8030) using a Shim-pack XR-ODS column (2 × 100 mm, 2.2 µm). A total of thirteen synthetic AHLs, ranging from C4 to C14, with various oxidation states, were used as reference AHLs for the analysis. Automatic MRM optimization was applied to each standard to determine the MRM transitions for subsequent sample analysis. A pair of MRM transitions was selected for each standard. The MRM transition that exhibited higher intensity was used for quantification analysis, while the other for confirmation of the AHL identity. In addition, MS full scan coupled with synchronized survey scan was employed to identify possible existence of other AHL structures with predicted *m/z* values.

In situ detection of AHLs in activated sludge samples

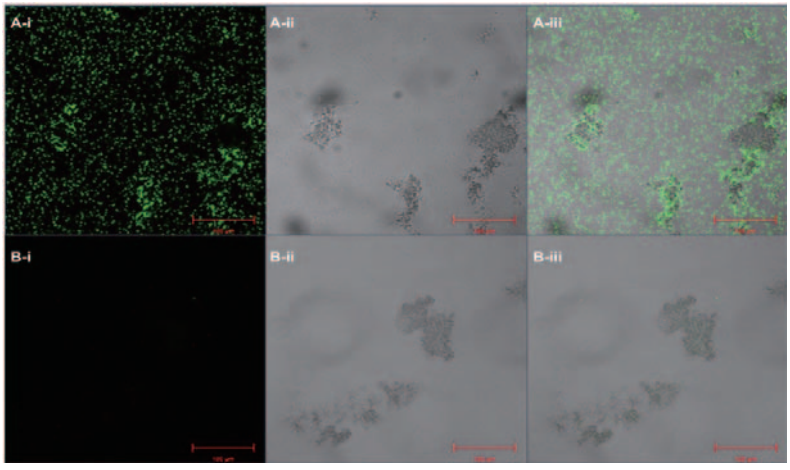

Fig. 2 Detection of AHLs present in situ in activated sludge samples using biosensor Escherichia coli pJBA357 (To be continued)

Fig. 2 (Continued). Co-incubation of live activated sludge (A) and heatinactivated sludge (B) with the biosensor for 4 hours at room temperature. The presence of AHLs is indicated by the expression of green fluorescent proteins by the biosensors, which can be visualized using confocal laser scanning microscope (CLSM).

MRM analysis of thirteen synthetic AHL standards

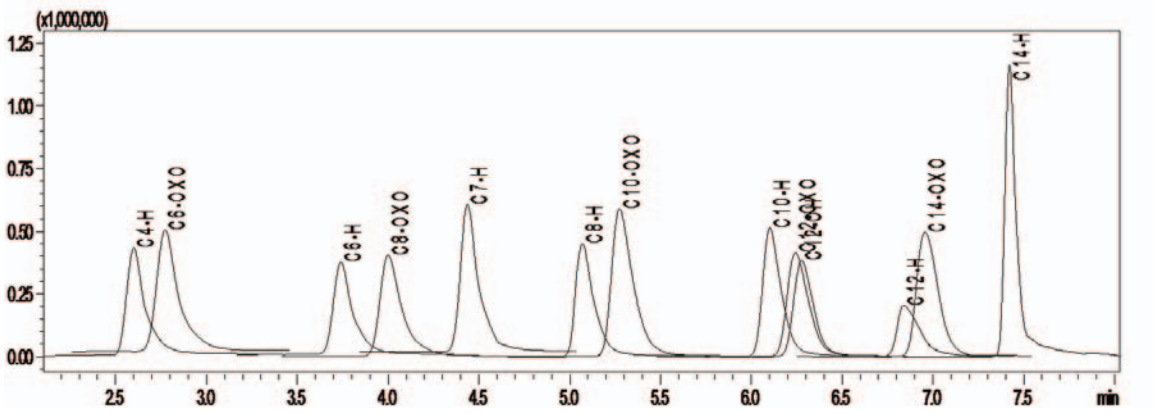

Fig. 3 MRM chromatogram for thirteen synthetic AHLs.

Table 1 Synthetic AHLs used as standard reference for analysis.

Peak	AHL identity	Peak	AHL identity
C4-H	N-butyryl-DL-homoserine lactone	C10-H	N-decanoyl-DL-homoserine lactone
C6-OXO	N-(3-Oxohexanoyl)-DL-homoserinelactone	C12-OXO	N-(3-oxododecanoyl)-L-homoserinelactone
C6-H	N-hexanoyl-DL-homoserine lactone	C12-OH	N-(3-hydroxydodecanoyl)-L-homoserinelactone
C8-OXO	N-(3-oxooctanoyl)-L-homoserine lactone	C12-H	N-dodecanoyl-DL-homoserine lactone
C7-H	N-heptanoyl-DL-homoserine lactone	C14-OXO	N-(3-oxotetradecanoyl)-L-homoserinelactone
C8-H	N-octanoyl-DL-homoserine lactone	C14-H	N-(3-oxotetradecanoyl)-L-homoserine lactone
C10-OXO	N-(3-oxodecanoyl)-L-homoserine lactone		

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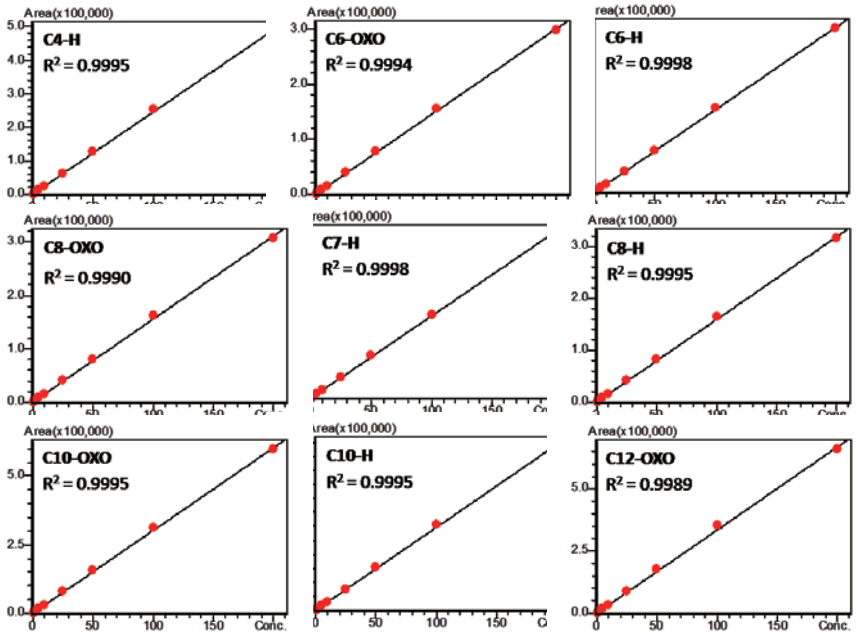

Fig. 4. Representatives of standard calibration curves for different synthetic AHLs spiked to the blank sample matrix. The limit of detection (LOD) for all the thirteen synthetic AHLs were found to be approximately 0.1 ppb to 1.0 ppb.

Case study 1 – Activated sludge samples (Ulu Pandan, Sg)

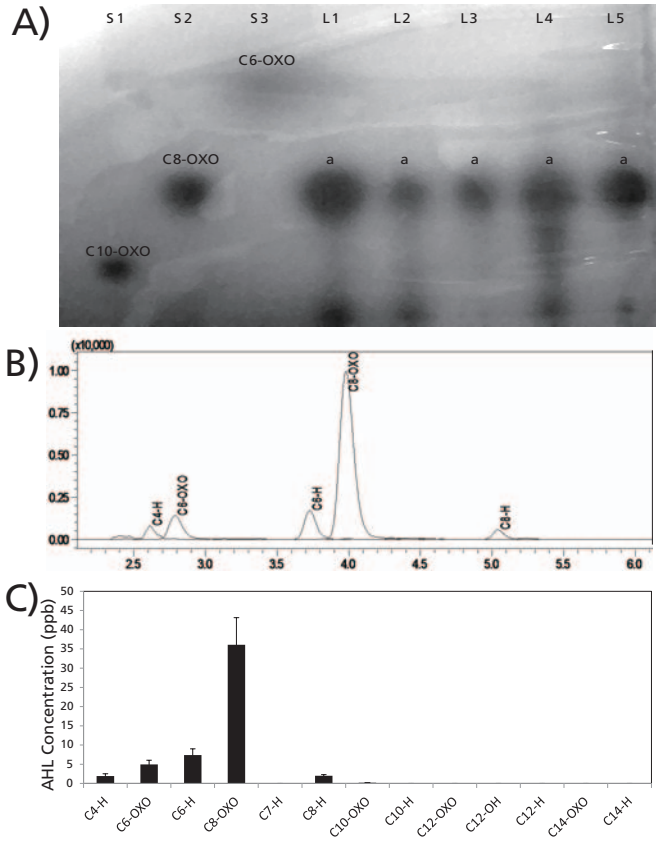

Fig.5 Detection of AHLs in activated sludge samples by two different approaches - Thin layer chromatography separation followed by detection using biosensor Agrobacterium tumefaciens A136 (A) and LCMS-8030 (B). Each of the AHLs identified in LCMS chromatogram (B) was quantified (C) based on the standard calibration curves generated in Fig. 4.

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Case study 2 – Bacterial isolates from the activated sludge

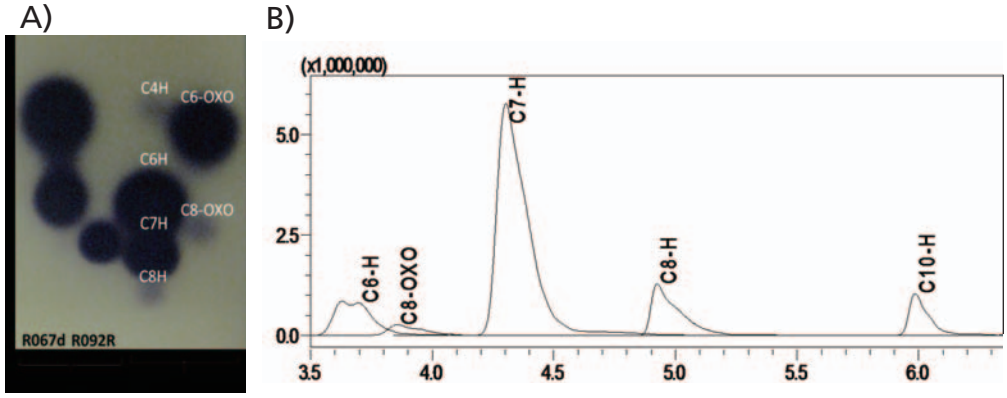

Fig. 6 Detection of AHLs in overnight culture of bacteria isolated from the activated sludge by two different approaches - Thin layer chromatography separation followed by detection using biosensor Chromobacterium violaceum CV026 (A) and LCMS-8030 (B). Note that only the AHL profile of bacteria isolate R092R is displayed in the LCMS chromatogram (B).

Case study 3 – Detection of AHL structures with predicted *m/z* values

MS full scan coupled with synchronized survey scan approach was employed to detect possible existence of other AHL structures beyond the thirteen synthetic AHL standards. A combination criteria of predicted retention time, and *m/z* values for both parent and product ions were included to confirm the identity of the compound.

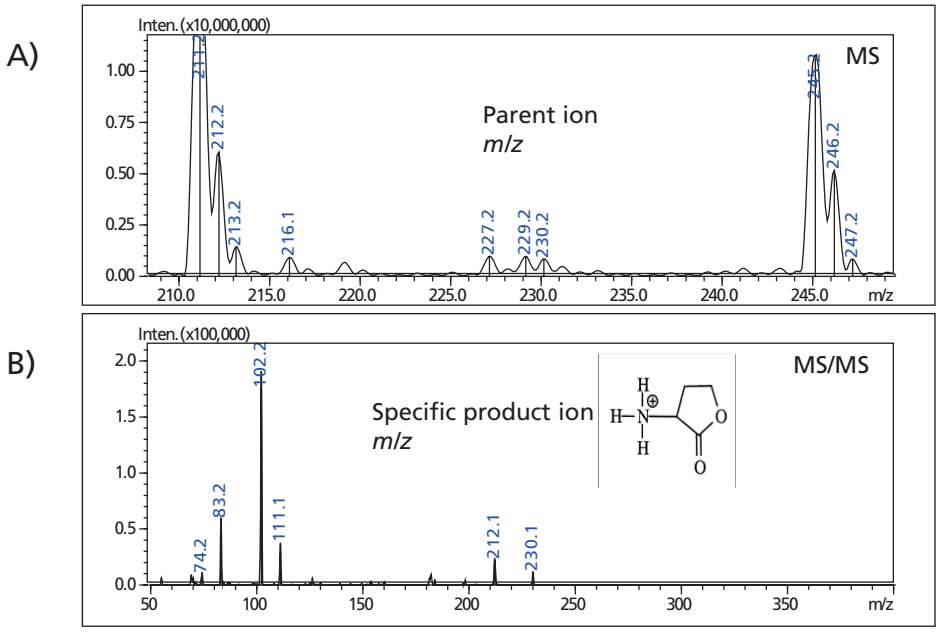

Fig 7 Detection of AHLs with predicted *m/z* values in R092R isolate using MS synchronized survey scan. Expected parent ion, *m/z* 230.2, was detected by MS scan mode (A) and MS fragmentation pattern of the target mass peak (B).

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Table 2 Detection of AHLs with predicted *m/z* values and retention time.

Peak	Retention time (min)	Parent ion (*m/z*)	Product ion (*m/z*)	Putative AHL identity
1	3.463	230.2	102.2	C7-OH
2	7.964	340.5	102.2	C16-H
3	7.340	354.5	102.1	C16-OXO

Conclusions

Advantages of using UHPLC/MS/MS over conventional biosensor approach in detection of AHL signaling molecules from complex environmental samples :

- Rapid, quantitative, high resolution, high throughput and highly sensitive
- Wide dynamic detection range
- Universal detection limit for all different AHL structures - Unbiased assessment
- Able to identify possible existence of AHL structures with predicted *m/z* values

Reference

1. Koutsoudis et al. 2006. Quorum-sensing regulation governs bacterial adhesion, biofilm development, and host colonization in Pantoea stewartii subspecies stewartii. Proc. Natl. Acad. Sci. USA 103:5983-5988
2. Wang et al. 2005. Rapid acyl-homoserine lactone quorum signal biodegradation in diverse soils. Appl. Environ. Microbiol. 71:1291-1299
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