第 53 卷 第 4 期
2016 年 7 月

土 壤 学 报
ACTA PEDOLOGICA SINICA

Vol. 53, No. 4
July, 2016

DOI：10.11766/trxb201511230492

信号分子*N*-酰基高丝氨酸内酯分析方法研究进展*

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摘 要 细菌能自发产生、释放特定的信号分子，并感知其浓度变化，调节微生物的群体行为，这一调控系统称为群体感应。革兰氏阴性菌的群体感应一般由*N*-酰基高丝氨酸内酯（Acyl-homoserine lactones，AHLs）这类信号分子介导。在简要总结AHLs分子及其衍生物结构的基础上，结合近年来国内外研究进展，对AHLs的提取、鉴定和检测等分析测试方法进行了综述，旨在为建立土壤中AHLs的检测方法以及深入研究AHLs信号分子在土壤中的环境行为奠定基础。

关键词 *N*-酰基高丝氨酸内酯；色谱-质谱；生物传感器；核磁共振；酶联免疫

中图分类号 O653；O656；O657 **文献标识码** A

随着微生物学的深入发展，群体感应现象逐渐被大家所熟知，许多微生物的生理特性和活动均与群体感应息息相关[1]。细菌群体感应（Quorum sensing，QS），也称自诱导，是细菌根据种群密度大小进行胞内或胞间信息交流，协调群体行为，并调控基因表达的一种机制。它利用信号分子（自诱导剂）在细胞间扩散，来感知自身和周围环境中其他菌群的数量变化，当信号分子浓度达到一定阈值时就启动菌体相关基因表达，调控相关生物学功能。革兰氏阴性细菌的群体感应一般是由LuxI/R型信息系统调控，并以酰基高丝氨酸内酯（Acyl-homoserine lactones，AHLs）作为自诱导剂[2-3]。许多革兰氏阴性菌均靠分泌AHLs进行交流和协调群体行为，例如病原细菌胞外酶和毒素的产生[4]、生物膜的形成[5-8]、生物发光[9-10]、色素产生[11]、抗生素形成[12]和细菌运动[13]等。笔者的最新研究表明，微生物信号传导还能促进有机污染物的降解[14]。

近几年来，研究细菌与细菌之间的信息交流已成为微生物学研究的热门领域[15]，科学家们希望能够针对细菌AHLs介导的QS 系统进行干扰和促进，从而达到有益于人类的目的。AHLs是革兰氏阴性菌QS 系统中的关键因子，如何快速、准确地鉴定并监测环境和细胞中AHLs的变化规律是研究群体感应的重要基础。本文系统综述、比较了目前用于定性、定量检测环境和细胞中AHLs信号分子的分析测定方法，以期为复杂介质（如土壤）中AHLs信号分子的测定提供指导。

1 酰基高丝氨酸内酯（AHLs）

不同细菌产生不同的AHLs，但它们均含有相

* 江苏省杰出青年基金（BK20150050）、国家重点基础研究发展计划（“973”计划）项目第五课题（2014CB441105）和国家自然科学基金项目（21277148，41301240，41271327）资助 Supported by the Jiangsu Provincial Foundation for Distinguished Young Scholars（No.BK20150050），the National Basic Research Program（“973”）of China（No. 2014CB441105），and the National Natural Science Foundation of China（Nos. 21277148，41301240，41271327）

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收稿日期：2015-11-23；收到修改稿日期：2016-01-15；优先数字出版日期（www.cnki.net）：2016-01-28

同的高丝氨酸内酯环，其差别在于*N*–酰基的碳链长度（碳原子数为4～18个，多为偶数，奇数中只有7）或3—碳位置的取代基（氢、羰基、羟基）不同[16]（图1）。侧链通过氨基化合链与内酯环的高丝氨酸的环状部分相连，形成酰化高丝氨酸内酯。侧链长短以及空间结构不同，AHLs的极性会有差异，一般情况下，酰化高丝氨酸内酯侧链越长，空间结构越不对称，其极性越大。此外，由于高丝氨酸内酯环具有亲水性，*N*–酰基的碳链具有疏水性，所以高丝氨酸内酯类化合物是一种具备水溶性、膜透过性的分子，可以自由出入细胞，在细胞间传递信息[1]。常见的AHLs及其理化性质见表1。

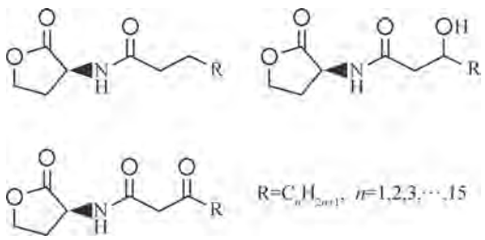

图1　常见酰基高丝氨酸内酯（AHLs）的结构

Fig. 1　Structure of common acy–homoserine lactones（AHLs）

表1　12种常见酰基高丝氨酸内酯（AHLs）信号分子的理化性质[1]

Table 1　Physicochemical properties of 12 commonly existed acyl-homoserine lactones（AHLs）

酰基高丝氨酸内酯 AHLs	简写 Acronym	分子式 Formula	分子量 Molecular mass（Da）	水溶解度 Water solubility（mg L^{-1}）	极性 Polarity	标化分配系数 Koc	log *P*[17]
N–butanoyl HSL	C_4–HSL	$C_8H_{13}NO_3$	171.2	1.40×10^5	5.80	0.02	0.03
N–（3–Oxohexanoyl）HSL	OXO–C_6–HSL	$C_{10}H_{15}NO_4$	213.2	1.04×10^5	–	0.09	0.14
N–hexanoyl HSL	C_6–HSL	$C_{10}H_{17}NO_3$	199.3	1.48×10^4	5.81	0.64	1.02
N–（3–Oxooctanoyl）HSL	OXO–C_8–HSL	$C_{12}H_{19}NO_4$	241.3	1.06×10^4	–	0.59	0.94
N–heptanoyl HSL	C_7–HSL	$C_{11}H_{19}NO_3$	213.3	4.76×10^3	6.00	0.95	1.51
N–octanoyl HSL	C_8–HSL	$C_{12}H_{21}NO_3$	227.3	1.53×10^3	6.19	1.24	1.97
N–（3–Oxodecanoyl）HSL	OXO–C_{10}–HSL	$C_{14}H_{23}NO_4$	269.3	1 072	–	0.71	1.70
N–decanoyl HSL	C_{10}–HSL	$C_{14}H_{25}NO_3$	255.4	1 550	5.90	1.86	2.96
N–（3–Oxo–dodecanoyl）HSL	OXO–C_{12}–HSL	$C_{16}H_{27}NO_4$	297.4	107.2	–	1.57	2.49
N–dodecanoyl HSL	C_{12}–HSL	$C_{16}H_{29}NO_3$	283.4	15.62	6.44	2.53	4.02
N–（3–Oxotetradecanoyl）HSL	OXO–C_{14}–HSL	$C_{18}H_{31}NO_4$	325.4	10.62	–	2.11	3.35
N–tetradecanoyl HSL	C_{14}–HSL	$C_{18}H_{33}NO_3$	311.5	1.56	6.54	3.21	5.09

注：log *P*表示AHLs在正辛醇和水溶液中的分配比 Note：log *P* stands for partition of AHLs between *n*-octanol alcohol and water

2　样品前处理

目前关于AHLs的提取检测主要针对溶液体系或者菌液。液液萃取（liquid liquid extraction，LLE）和固相萃取（solid phase extraction，SPE）是从溶液中分离AHLs最常用的两种方法[18]。液液萃取是提取AHLs最成熟、最常用的方法[19]。萃取剂主要包括二氯甲烷[20]、氯仿[21]、乙酸乙酯[22]和乙醚[23]等（表2）。其中以二氯甲烷和氯仿的提取效果最好，萃取效率也几乎相同。不过，萃取率随着AHLs极性的增加而减小。笔者研究发现，采用乙酸乙酯萃取时，随着AHLs的碳原子数与log *P*值的增加，萃取率先从C_4–HSL开始上升，至C_8–HSL达到最大值，之后逐渐下降[1]。这与Morin等[24]采用二氯甲烷提取菌液中AHLs的规律类似。对菌液的分析，首先离心将微生物或颗粒物分离，然后萃取；如果需要和菌一起分析，就直接萃取，萃取后的样品可能含有蛋白等杂质，可用PES聚醚砜滤膜过滤纯化[19]；纯化后的样品经浓缩、定容后测定。

固相萃取是另一种高效萃取方法，该方法兼具浓缩和纯化功能。Li等[25]采用C_{18}柱研究发现与液

液萃取相比，固相萃取能将灵敏度提高两至十倍。使用固相萃取柱之前，通常要用甲醇/水活化填料，待萃取水样也要加入少量乙腈调节极性。样品经抽滤过柱后，采用异丙醇/正己烷（*v*/*v*，75/25）洗脱吸附的AHLs，然后氮吹浓缩近干后，溶解于乙酸乙酯或乙腈后进行定性定量分析[25]。

表2 酰基高丝氨酸内酯（AHLs）萃取剂

Table 2 Extractants of acyl-homoserine lactones（AHLs）

目的细菌 Strains	提取剂 Extraction agent	溶剂 Solvent	参考文献 Refs
*C. violaceum*ATCC 31532	二氯甲烷 Dichloromethane	乙腈 Acetonitrile	[20]
*R. leguminosarum*bv. viciae 248	氯仿-甲醇 Chloroform-methanol（*v*/*v*，97.5∶2.5）	乙腈-水溶液 Acetonitrile-water（*v*/*v*，50∶50）	[21]
P. aeru-ginosa strain PAO214	酸化乙酸乙酯 Ethyl acetate	乙腈-水溶液 Acetonitrile-water（*v*/*v*，90∶10）	[26]
P. aeruginosa	乙酸乙酯 Ethyl acetate	甲醇 Methanol	[22]
Vibrio vulnificus	二氯甲烷 Dichloromethane	乙腈（色谱纯） Acetonitrile（chromatograph）	[24]
E.coli strains	乙酸乙酯 Ethyl acetate	乙酸乙酯 Ethyl acetate	[23]

3 酰基高丝氨酸内酯的鉴定

未知信号分子的鉴定对了解微生物群落间的信息交流起着至关重要的作用。AHLs的结构鉴定包括色谱-质谱联用、毛细管区带电泳与质谱联用、傅里叶变换离子回旋共振质谱和核磁共振等技术（表3），其中，前两类方法既可定性又可定量。

3.1 色谱-质谱联用

高效液相色谱-质谱法和气相色谱-质谱法一般采用反相高效液相色谱-质谱联用来分析AHLs，以水/甲醇或水/乙腈为流动相，用C_{18}硅胶柱进行分离，质谱条件选用电喷雾离子源，采用正离子扫描模式[27-29]。也可以采用气相色谱-质谱联用测定AHLs，通常以氦气作为载气，程序升温从100℃至280℃，在20 m长的毛细管柱中分离样品，以选择性离子检测模式（m/z143）进行分析[1]。Charlton等[30]研发了一种定量检测3-oxo-HSLs的气质联用方法，他们将3-oxo-HSLs转化为特定的衍生物，通过气相色谱-质谱的方法检测。由于AHLs的低挥发性和在高温进样口中的不稳定性，气质联用较液质联用更难鉴定。然而，高效液相色谱串联质谱会受到液体流动相的限制，如果流动相的流型有变化，被分离物质的扩散和滞留均会显著地导致色谱峰加宽，柱效率降低，并且梯度洗脱参数设定较为复杂，检测成本高。

纳升级液相色谱串联质谱 Frommberger研发了一种采用纳升级液相色谱串联质谱（nano-liquid-chromatography-mass/mass spectrometry，nano-LC-MS/MS）鉴定菌液中AHLs的方法[44]，他们在nano-LC和MS之间加入一个微电喷射界面，目标化合物首先在填有反相硅胶颗粒的毛细管柱的端口浓缩，接着通过有机流动相进行洗脱和选择性分离，样品预浓缩类似于小规模的固相萃取，相对于流动相（甲醇/水（*v*/*v*，80/20））样品溶剂（甲醇/水（*v*/*v*，30/70））的极性有所提高，从而能够有选择性地和高精度地检测AHLs。该方法分离效果好，灵敏度高，达1 μg L^{-1}使用的毛细管柱容易生产，且只需要极少量的溶剂就可完成测定，经济可行性高。

毛细管区带电泳串联质谱（capillary zone electrophoresis/mass spectrometry，CZE/MS）也可分析AHLs，而检测基于质谱，为了避免由于离子

表3　酰基高丝氨酸内酯（AHLs）测定方法[17]

Table 3　Methods developed for determination of acyl-homoserine lactones（AHLs）compounds

方法 Method	基质 Substrate	产物鉴定 Product determination	样品处理 Sample treatment	参考文献 Refs
反相高效液相色谱-质谱法 RP-HPLC-MS	*E.Coli*，*P.stewartii* *P.aeruginosa*	oxo-C_6，C_6-，C_{12}-HSL	固相萃取 SPE	[31]
生物传感器 Biosensor	Cystic fibrosis patients	C_4-，C_6-，C_{10}-，C_{12}-，oxo-C_6-，3-oxo-C_{10}-HSL，3-oxo-C_8-	液液萃取（二氯甲烷）和液相色谱联用 LLE plus LC	[26]
反相高效液相色谱-质谱法 RP-HPLC-MS	*V.Anguillarum*， infected， rainbow trout	OH-C_6-，C_6-，oxo-C_{10}-HSL	液液萃取（二氯甲烷）和液相色谱联用 LLE plus LC	[32]
气相色谱-质谱 电喷雾电离-质谱-质谱 GC-MS，ESI-MS-MS	*Halomonas* specie *Sinorhizobium meliloti*	C_4-，C_6-，C_8-，C_{12}-，oxo-C_{10}-HSL	液液萃取（二氯甲烷）和高效液相色谱联用 LLE plus HPLC	[33-34]
气相色谱-荧光/质谱 GC-LIF，GC-MS	Bacterial supernatant	C_6-HSL	液液萃取（二氯甲烷）和高效液相色谱联用 LLE plus HPLC	[29]
反相高效液相色谱-PDA/质谱 RP-HPLC-PDA and MS	Bacterial supernatant	C_4-，C_6-HSL	液液萃取（二氯甲烷）和高效液相色谱联用 LLE plus HPLC	[35]
生物传感器 Biosensor	*Agrobacterium tumefaciens*	oxo-C_6-HSL，C_6-HSL	固相萃取 SPE	[36]
气相色谱-质谱 GC-MS	*Burkholderia cepacia*	C_4-，C_6-，C_7-，C_8-，C_{10}-，C_{12}-，C_{14}-HSL	液液萃取（氯仿） LLE	[37]
气相色谱-质谱 GC-MS	Marine isolate	C_4-，oxo-C_6-，C_8-，C_{12}-HSL	五氟卞肟衍生化 Derivatization with pentafluorobenzyloxime	[38]
高效液相色谱-质谱 HPLC-MS	*Vibrio vulnificus* *P.aeruginosa*	C_4-，C_6-，C_7-，C_8-，C_{10}-，C_{12}-，C_{14}-，oxo-C_6-，oxo-C_8-，oxo-C_{10}-，oxo-C_{12}-，oxo-C_{14}-	液液萃取（氯仿） LLE	[24]
反相高效液相色谱-高分辨质谱 RP-HPLC-HRMS	Fillets of cod	Hydroxyl-C_8-，C_4-，C_6-，C_8-，oxo-C_8，oxo-C_{10}	液液萃取（乙酸乙酯） LLE	[39]
反相高效液相色谱-高分辨质谱 RP-HPLC-HRMS	Spoiled meat	oxo-C_6-，C_6-，oxo-C_8-，C_8-HSL	液液萃取（氯仿）和薄层层析联用 LLE plus TLC	[40]
反相高效液相色谱- 大气压化学电离质谱 RP-HPLC-APCI-MS	Soil and Bacterial supernatant	C_4-，C_6-，C_7-，C_8-，C_{10}-，C_{12}-，C_{14}-，oxo-C_6，oxo-C_{12}-HSL		[41]
反相高效液相色谱- 电喷雾电离质谱 RP-HPLC-ESI-MS	Bacterial biofilm	oxo-C_{10}-HSL	液液萃取（乙酸乙酯） LLE	[42]
反相高效液相色谱-高分辨质谱 反相高效液相色谱-二极管阵列 RP-HPLC-APCI-MS RP-HPLC-DAD	Standard solution	C_6-，oxo-C_6-，oxo-C_8-HSL		[43]

续表

方法 Method	基质 Substrate	产物鉴定 Product determination	样品处理 Sample treatment	参考文献 Refs
纳升级液相色谱-电喷雾电离质谱 Nano-LC-ESI-MS	*Burkholderia cepacia*	C_4-, C_6-, C_7-, C_8-, C_{10}-, C_{12}-, C_{14}-HSL	液液萃取（氯仿） LLE	[44]
毛细管区带电泳-电喷雾电离质谱 CZE-ESI-MS	*Burkholderia cepacia*	C_4-, C_6-, C_7-, C_8-, C_{10}-, C_{12}-, C_{14}-HSL	液液萃取（氯仿） 水解固相萃取 LLE plus SPE	[45]
胶束电动毛细管色谱-电喷雾质谱 PF-MEKC-ESI-MS	*Burkholderia cepacia*	C_4-, C_6-, C_7-, C_8-, C_{10}-, C_{12}-, C_{14}-HSL	液液萃取（氯仿） LLE	[46]
高效液相色谱-质谱 HPLC-HRMS	Spoiled bean sprouts	oxo-C_6-HSL	固相萃取 SPE	[47]

抑制导致电喷雾灵敏度下降，特别在毛细管色谱柱中注入表面活性剂以提高灵敏度。CZE用来分析由AHLs碱性水解产生的丝氨酸分子。碱性条件下，丝氨酸的羧酸基能够完全去质子化，从而实现分离。通常向含有AHLs的样品中加入1 mol L^{-1} NaOH进行水解，室温下培养15 min，将水解产物加载到CZE检测器[45]。由于丝氨酸作为阴离子被分离，因此，丝氨酸的碳链越长（分子越大），移动越快，其分离顺序与高效液相色谱正好相反。应用该方法分析*Burkholderia* sp. Mmi1537产生的AHLs，以C_7-HSL为内标，检测到C_8-HSL、C_{10}-HSL和C_{12}-HSL[45]。CZE/MS提供了一种定量区分高丝氨酸内酯与水解产物的方法。

3.2 傅里叶变换离子回旋共振质谱及其与超高效液相色谱联用

采用傅里叶变换离子回旋共振质谱（Fourier transform ion cyclotron resonance/mass spectrometry，FTICR/MS）鉴定AHLs结构的方法最先由德国的P. Schmitt-Kopplin课题组研发[48]，采用基于芯片的电喷雾离子系统，在正离子检测模式下发生离子化。AHLs作为质子化形态［M+H］$^+$和钠化合物［M+Na］$^+$一起测定[14, 49]。FTICR/MS可以达到百万分之一的精确度，对于未知小分子（大约 500 Da）结构式的鉴定非常可靠。当精确度低于百万分之一时，基于同位素丰度的正交滤波器也能够确定分子结构式，利用多种元素（C，H，N，S，O，P和F，Cl，Br，Si）的同位素峰，能够鉴定出许多化合物。高磁场FTICR/MS（例如12 特斯拉）能够可靠鉴定复杂样品中的代谢物结构。质谱在宽带模式下检测，其半峰全宽值超过2 00000，在高分辨模式甚至可以达到1 000000。然而，FTICR/MS不容易区分等比重离子。

超高效液相色谱（Ultra high performance liquid chromatography，UPLC）基于小的多孔颗粒和高压原理，也常用来分离定量测定AHLs，具有分辨率高、柱效好、峰窄及速率高等优点。通常用光电管矩阵检测器在210或197 nm进行测定[20]。使用C_{18}反相分离柱，乙腈/水或者甲醇/水作为流动相分离AHLs。Morin等[24]比较了这两种洗脱液后发现，使用甲醇时最先出的峰大些，但是分离效果和信号强度相对更好。当使用线性梯度的甲醇/水溶剂作为流动相，洗脱峰会变窄[22]。AHLs的侧链影响其极性，而极性又决定色谱保留时间。研究表明，log *P*值（*P*为保留时间）与侧链长度成比例[17]。因此，可以通过色谱保留时间获得一个未知的AHLs分子的侧链长度。

FTICR/MS与UPLC联用能够大大增强鉴定未知化合物的能力。为了将UPLC的速度效率和FTICR/MS较高的灵敏度有机结合，Li等[48]采用一个96孔板连接，将UPLC分离的样品分别收集，然后采用FTICR/MS测定。

3.3 核磁共振

核磁共振是鉴定有机化合物结构的一种重要方法，有机化合物中的碳、氢链接在附近的原子核上，在不同的化学环境下共振断裂，以单峰、双峰、三重峰等形式出现。核磁共振已用于检测

AHLs[12]、DKP[50]（二酮哌嗪类化合物）等信号分子。由于越来越多的研究发现，微生物释放的AHLs分子，有些含有双键结构[14]，要确定双键在碳链上的位置，核磁共振分析可以解决此问题。

4　酰基高丝氨酸内酯的定量分析

定量检测AHLs的方法包括：生物传感器、薄层层析法、放射性同位素示踪法、酶联免疫法、比色法和色谱法等。其中，生物传感器、薄层层析、色谱法和酶联免疫法是常用分析方法，但均有自己的优缺点。生物传感器检测精度达飞摩尔级，但是每种AHLs均需要特定的菌株和质粒，并且需要一天的时间才能完成测定。薄层层析的精度不高，只能用来测定较高浓度的AHLs。酶联免疫法和比色法简单、方便、快捷，但不能区分不同的AHLs。色谱可以同时定量检测多种AHLs，但是样品需要进行提取和纯化，前面已经介绍，这里不再阐述。

4.1　生物传感器法

生物传感器广泛用于AHLs的检测。由于调控AHLs的启动子由外源AHLs控制，这些生物传感器往往自身不能合成AHLs。但是，在外源AHLs的作用下，它们能够发光、变色或表达β–半乳糖苷酶活性，因而具有检测AHLs的功能。根据不同菌株含有不同AHLs启动子能够非常高效地检测不同链长的AHLs（表4）。据Steindler等[51]报道，浓度

表4　酰基高丝氨酸内酯（AHLs）生物传感器[18]

Table 4　Biosensors for determination of acyl–homoserine lactones

生物传感器 Biosensor	来源 Source	群体感应系统 Quorum sensing	报告系统 Reporting system	检测的AHLs Detected AHLs	参考文献 Refs
CV026	*C.violaceum*	CviI/R	Violacein pigment	C_6–3–oxo–HSL C_8–HSL，C_4–HSL C_8–3–oxo–HSL	[20]
pSB401	*E. coli*	LuxI/R	*LuxCDABE*	C_6–HSL，C_8–HSL C_8–3–oxo–HSL	[54]
pHV2001–	*E. coli*	LuxI/R	*LuxCDABE*	C_6–AHLs，C_8–HSL C_8–3–oxo–HSL	[3]
pZLR4	*A.tumefaciens NT 1*	TraI/R	β–galactosidase	All 3–oxo–HSL $C_6 \sim C_{14}$–HSL $C_6 \sim C_{10}$–3–hydroxxy–HSL	[55]
pCF218+ pCF372	*A.tumefaciens WCF47*	TraI/R	β–galactosidase	As above with more sensitivity	[53]
pJZ384+ pJZ410+ pJZ372	*A.tumefaciens KYC55*	TraI/R	β–galactosidase	As above with more sensitivity	[41]
pSB403	*V. fisheri*	LuxI/R	*LuxCDABE*	C_6–HSL，C_8–HSL C_6–3–oxo–HSL	[56]
pSB536	*E. coli*	AhyI/R	*LuxCDABE*	C_4–HSL	[15]
pAL101	*E. coli*	RhlI/R	*LuxCDABE*	C_4–HSL	[57]
pSB1075	*E. coli*	LasI/R	*LuxCDABE*	C_{10}–3–oxo–HSL C_{12}–HSL	[54]
pSB406	*P. aeruginosa*	RhlI/R	*LuxCDABE*	$C_4 \sim C_{14}$–3–oxo–HSL $C_4 \sim C_{12}$–3–HSL	[54]
pKR–C12	*P. aeruginosa*	LasI/R	*gfp*	$C_{10} \sim C_{14}$–3–oxo–HSL $C_{10} \sim C_{12}$–3–HSL	[21]
pAS–C8	*B. cepacia*	CepI/R	*gfp*	$C_{10} \sim C_{12}$–3–oxo–HSL $C_6 \sim C_{12}$–HSL	[21]

低至20 nmol L^{-1}的AHLs也足以引起检测基因的表达，而且，检测具有高度选择性：含有pAS-C8报告基因质粒的*P. putida* F117检测3-oxo-C_{12}-HSL的灵敏度较C_{12}-HSL高100多倍。不过，由于每种生物传感器只能检测特定的AHLs分子，因此，通常需要多种生物传感器来检测一种微生物产生的一系列AHLs。

生物传感器是检测AHLs是否存在的最简单、有效的工具，该方法可以直接通过肉眼检测出AHLs，与其他方法结合可以定量检测AHLs的浓度。然而，它不能区分AHLs的侧链长度与不同的取代基，因而无法区分各个AHLs。此外，由于基质的相互作用，也可能导致传感器错误指示。当生物传感器应用于生物体原位检测时并不总是有效，例如，许多细菌均确定产生了AHLs，然而采用生物传感器检测却显示阴性结果[52]。因此，该方法常常与其他方法联合使用，比如菌落法[53]或薄层层析法。菌落法：将培养液上清液或者样品萃取物涂布在覆盖有一层传感器的琼脂培养基上面，培养过夜后，由于外源AHLs的加入，围绕菌落呈现出一个颜色圈。

4.2 薄层层析法

通常采用C_{18}反相薄层层析板，将层析板垂直放置在含有样品的玻璃室中，不同极性的AHLs样品以不同的速率移动，在薄层层析板上，一系列的酰基高丝氨酸内酯会沿着移动轨迹得以分离。分离后的样品可采用以下三种方法测定：

生物传感器：将一种接种AHLs感应菌的软琼脂覆盖在薄层层析板表面，30℃下培养16 h，AHLs存在的区域会呈现亮点或颜色变化，对紫色杆菌素的产量或β-半乳糖苷酶的活性进行定量分析。通过AHLs标准样品获得其标准曲线，利用薄层层析板上斑点区域计算AHLs的残留浓度[54]。

硫酸法：待薄层层析板上的样品和标准品干燥后，将重铬酸钾溶液（该溶液溶于硫酸溶液（*v*/*v*，40/60））喷在层析板上，200℃下加热10 min，烧焦的炭黑沉积物明显完整的遗留下来。通过比较AHLs样品和标准品的位移比，能初步确定AHLs的结构信息[58]。然而，该方法极具破坏性，检测后的AHLs不能复原，所以不能用来分离和纯化AHLs。

联合色谱分析：将薄层层析板上有反应的区域刮下来，用二氯甲烷或者乙酸乙酯萃取，然后浓缩，可用于进一步色谱/质谱分析[59]。

4.3 放射性同位素示踪法

微生物产生的AHLs浓度通常很低，而且由于生物膜的存在，AHLs的提取非常困难。Schaefer等[60]发明了一种放射性同位素方法来检测AHLs，其原理是活体细胞吸收的放射性蛋氨酸会与AHLs反应。其步骤为：将测试菌在不含蛋氨酸的基质中培养至稳定期初期，加入体积比为1%的^{14}C-蛋氨酸，培养一定时间，放射性蛋氨酸会结合AHLs形成酰基高丝氨酸内酯—蛋氨酸螯合物，可以直接取上清液，然后与液体闪烁溶剂反应，采用液体闪烁计数仪定量测定^{14}C。该分析方法不受环境介质的干扰，不需复杂的样品前处理。不过，该方法只能测定AHLs混合物的总量，无法区分AHLs的具体组成。

4.4 比色法

Yang等[61]发明了一种测定AHLs的比色法，该方法简单、快速、高效，可以测定AHLs的浓度，而且只需要极少量的样品（20～50 μl），它能追踪近乎1 nmol L^{-1}的AHLs，非常接近高效液相色谱的检测精度。此方法根据由苯酚形成的酯分子分析方法改进得来[62]，将样品放置在96孔的层析板上，加入体积比为1∶1的羟基胺（2 mol L^{-1}）和氢氧化钠（3.5 mol L^{-1}）混合物，混匀。紧接着将同等剂量的氯化铁（10%溶于4 mol L^{-1} HCl）和酒精（95%）混合液添加至样品溶剂中，样品中含有AHLs的区域会呈现暗褐色斑点，但在某些情况下，斑点颜色会受化合物浓度影响而转变为黄色。利用分光光度法在520 nm波长下定量检测各种内酯含量，会发现光密度值和AHLs摩尔浓度之间存在着良好的线性关系，并且重现性非常好。然而，由于光密度值适用于任何含有酯基的有机化合物。因此，任何出现在酰基高丝氨酸内酯样品中的污染组分，均会使检测结果偏高。

4.5 酶联免疫法

酶联免疫法（Enzyme-linked immunosorbent assay，ELISA）是检测AHLs的一种极其快速、经济有效的方法，需要的样品体积非常少（100 μl），检测精度非常低，即使有菌存在，对结果也几乎没有影响。具体方法：以单抗最佳包被浓度包被酶标板，每孔200 μl G蛋白溶液（pH=9.6，浓度为2 μg ml^{-1}，溶于50 mmol L^{-1}的碳酸盐缓冲液），4℃过夜。第二天，弃包被液，洗涤酶标板，拍

干后加入封闭液，每孔加入150μl的单克隆抗体（monoclonal antibody，mAb）溶液（HSL1/2–2C10）反应2 h，再次洗涤酶标板，拍干后每孔添加100 μl待检溶液（1h）。将预先准备好的HSL1–HRP（或HSL3–HRP）溶液直接添加到酶标板的微孔中，每孔50 μl，混合震荡培养1 h，洗涤酶标板，加入新配的四甲基联苯胺溶液150 μl，37℃下显色5～25 min，最后每孔添加50 μl的2 mol L^{-1} H_2SO_4，终止反应[63]。酶示踪模式见图2。然后用含有吐温20的磷酸盐缓冲液清洗，测定450 nm波长的吸光度值，根据标准曲线即可确定AHLs的浓度。

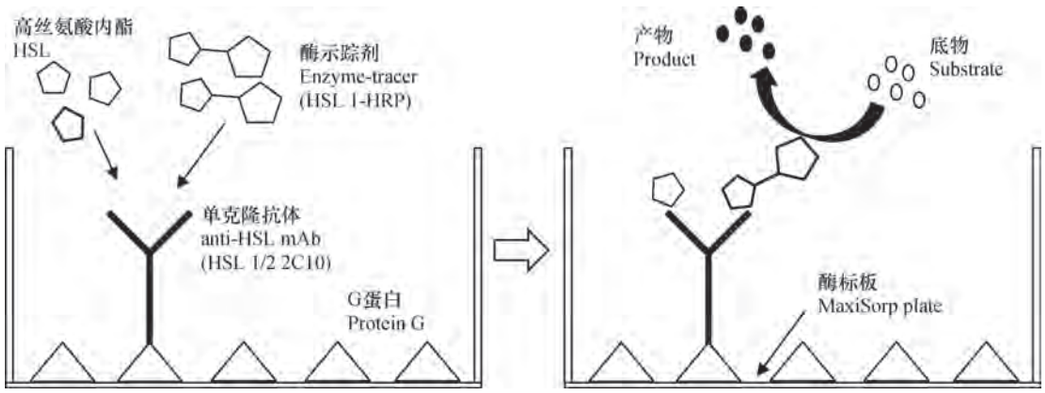

图2　酶联示踪模式下的酶联免疫测定[63]

Fig. 2　Determination of ELISAs in the enzyme tracing mode

5　展　望

N–酰基高丝氨酸内酯的测定对理解微生物间信号交流非常重要，然而，AHLs分子结构组成差异小，AHLs的浓度又极低，给定性与定量检测带来巨大挑战。本文综述了近年来AHLs的分析测定方法，比如：生物传感器、色谱分析、核磁共振、红外光谱等，每种方法均有优缺点。因此，采用多种方法联合优势互补来有效测定AHLs。

此外，目前的方法主要是针对纯溶液体系。在复杂环境（土壤、沉积物等）中，地球化学和物理条件的变化影响着微生物的生存环境，进一步影响着AHLs的释放与迁移变化[64]。复杂介质中的有机、无机化合物也对AHLs的纯化与检测带来极大的挑战，并且对于复杂介质中AHLs测定方法仅有极少数文献报道。Burmølle等[65]曾借助功能微生物*E. coli* MC4100/pAHL–GFP来测定土壤中*Serratialiquefaciens*产生的C_{10}–HSL。Steidle等[66]基于导入绿色荧光蛋白的菌株测定西红柿根际土壤中的AHLs，通过AHLs的动态变化来研究根际土壤中微生物间的信息交流。但由于功能微生物的培养、存活需要特定的条件，测定过程也需要较长的时间，并且每种功能菌只能够测定特定的AHLs，从而导致以上方法不能够被广泛应用。然而，自然界中微生物的信号传递与交流无处不在，尤其是土壤生态系统，每克土壤含有100亿个微生物细胞，是一个微生物非常活跃的场所，因此，发展复杂介质中既能高效快速，同时又能定性定量检测多种AHLs的方法，并且测定过程不受环境等外界条件的制约，对阐释这些环境中微生物的群体感应行为与机制具有重要作用。

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Advance in Study on Methods for Analysis of *N*-Acyl-Homoserine Lactones

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Abstract　With the development of microbiology, Quorum sensing (QS), closely related to many physiological characteristics and activities, is getting more and more attention. QS in bacteria is a kind of mechanism for intracellular or intercellular communication in response to variation of the community in density, aiming at coordinating their population behavior and controlling gene expression. QS of Gram-negative bacteria is generally controlled by the LuxI/R-type information system, using *N*-acyl-homoserine lactones (AHLs) as signal molecules to regulate diverse physiological processes such as generation of exoenzyme and toxin by pathogenic bacteria, formation of biomembrane, bioluminescence, biosynthesis of antibiotic, bacteria motility and even degradation of organic pollutants.

How to rapidly and accurately detect and monitor variation of AHLs in environment and cells is an important basis for the study on quorum sensing. Therefore, an overall review is presented here on AHLs as representative signal molecules, summarizing their characteristics, structures, derivatives and different analyzing methods as well.

As the concentration of AHLs in the cell and the environment is usually very low, pre-concentration is necessary for its determination. For pre-treatment of liquid samples, Liquid-liquid extraction and solid-phase extraction are two common methods used to isolate AHLs from liquid matrix. The former is the most widely used one, while the latter has dual functions, concentration and purification, simultaneously. The identification of unknown signal molecules plays a vital role in understanding the information communication between microfloras. To identify structures of AHLs, the techniques of chromatograph-mass spectrometer, capillary zone electrophoresis/mass spectrometry (CZE/MS), Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS) and nuclear magnetic resonance spectroscopy (NMR) are commonly used. To quantitatively analyze AHLs compounds, the techniques of biosensors, thin layer chromatography, high performance liquid chromatography (HPLC), radioactive isotope tracer and enzyme-linked immunosorbent assay (ELISA) are used. Each technique has its own advantages and disadvantages. Biosensors are very sensitive, however the analysis of each type of AHLs needs specific strain and plasmid. Thin layer chromatography, with a lower sensitivity, is only used to detect high concentration of AHLs. Radioactive isotope tracer is immune to interference of the external environment and hence makes the pretreatment of samples simple, however, this method can only be used to determine the total, rather than specific components of AHLs. HPLC is the most widely used to measure the content and structure of AHLs, but the samples for determination need to be pretreated for purification. Being low in cost, easy to operate and high in accuracy, ELISA has turned out to be a promising method, nevertheless, it could not be used to determine molecular structure of AHLs.

The determination and characterization of AHLs are very important to understand inter-microbial signal

communication, however it is still a big challenge to determine AHLs qualitatively and quantitatively because they do not vary much in molecular structure and are often very low in concentration. Although the above mentioned analytic methods are quite high in sensitivity and selectivity, each has its own disadvantage. Therefore, it is advisable to combine some of these methods to bring their advantages into full play and complement each other in determining AHLs. At the same time, how to combine these methods into one that is capable of determining a variety of AHLs efficiently and rapidly shall be the focus of the researches in future.

On the other hand, the current methods are mainly focused on aqueous solution. In complex natural environments, such as soil and sediment, the existence of organic and inorganic compounds simultaneously presents a great challenge to extraction, purification and determination of *N*-acy-homoserine lactones. It is necessary to develop some efficient methods for qualitatively and quantitatively measuring AHLs in complex matrices. The determination will play an important role in unveiling the mechanism and action of microbial quorum sensing in the natural environment, especially the soil ecosystem.

Key words *N*-acyl-homoserine lactones; Chromatography-mass spectrometry; Biosensor; Nuclear magnetic resonance; Enzyme-linked immunosorbent

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