N-acylhomoserine lactonase-producing *Rhodococcus* spp. with different AHL-degrading activities

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Abstract

N-acylhomoserine lactones (AHLs) are conserved signal molecules that control diverse biological activities in quorum sensing system of Gram-negative bacteria. Recently, several soil bacteria were found to degrade AHLs, thereby interfering with the quorum sensing system. Previously, *Rhodococcus erythropolis* W2 was reported to degrade AHLs by both oxido-reductase and AHL-acylase. In the present study, two AHL-utilizing bacteria, strains LS31 and PI33, were isolated and identified as the genus *Rhodococcus*. They exhibited different AHL-utilization abilities: *Rhodococcus* sp. strain LS31 rapidly degraded a wide range of AHLs, including *N*-3-oxo-hexanoyl-L-homoserine lactone (OHHL), whereas *Rhodococcus* sp. strain PI33 showed relatively less activity towards 3-oxo substituents. Coculture of strain LS31 with *Erwinia carotovora* effectively reduced the amount of OHHL and pectate lyase activity, compared with coculture of strain PI33 with *E. carotovora*. A mass spectrometry analysis indicated that both strains hydrolyzed the lactone ring of AHL to generate acylhomoserine, suggesting that AHL-lactonases (AHLases) from the two *Rhodococcus* strains are involved in the degradation of AHL, in contrast to *R. erythropolis* W2. To the best of our knowledge, this is the first report on AHLases of *Rhodococcus* spp.

Introduction

Quorum sensing is a regulatory mechanism used by bacteria to coordinate the behavior of their community in response to their population density (Fuqua *et al*., 1994; Salmond *et al*., 1995; Swift *et al*., 1996). Bacteria recognize the changes in their population density by sensing the concentration of signal molecules which are accumulated as bacterial cells proliferate. While chemically distinct families of signal molecules have been described, many Gram-negative bacteria use *N*-acylhomoserine lactones (AHLs) as the signal molecules. Signal specificity of AHLs is determined by the length and the substitution of an acyl side chain linked to the homoserine lactone ring by an amide bond (Eberhard *et al*., 1981; Pearson *et al*., 1994). The perception of AHLs relies on one or more sensor proteins, LuxR-type transcriptional regulators (Zhu & Winans, 1999; Welch *et al*., 2000), whose complex with AHLs controls the expression of diverse biological activities, including bioluminescence (Hastings & Nealson, 1977; Nealson & Hastings, 1979), antibiotic synthesis (Bainton *et al*., 1992; Wood *et al*., 1997), biofilm formation (Davies *et al*., 1998; Hentzer *et al*., 2003), swarming (Eberl *et al*., 1996), and the production of virulence factors (Jones *et al*., 1993; Passador *et al*., 1993; de Kievit & Iglewski, 2000).

As AHL-mediated quorum sensing has been shown to be involved in pathogenesis and biofilm formation, the interference of quorum sensing, referred as quorum quenching, has recently received a great deal of attention. Among the quorum quenching strategies, the potent effect of enzyme-based AHL degradation has been identified in a wide diversity of soil bacteria, including Proteobacteria (Leadbetter & Greenberg, 2000; Huang *et al*., 2003; Lin *et al*., 2003), low G+C Gram-positive bacteria (Dong *et al*., 2000; Lee *et al*., 2002), and high G+C Gram-positive bacteria (Park *et al*., 2003, 2005; Uroz *et al*., 2003). The AHL-degrading enzymes fall into two groups according to the cleavage site of AHL. AHLases, identified in the *Bacillus cereus* group, degrade AHLs by hydrolyzing the lactone ring of AHLs and produce corresponding acylhomoserine molecules (Dong *et al*., 2001). In addition to quorum quenching, the AHL lactonase, AhlD, from *Arthrobacter* IBN110 has recently been found to be involved in the utilization of AHLs as a nutrient source (Park *et al*., 2003). On the other hand, the

amide bond of AHLs is broken by AHL-acylases, thereby releasing homoserine lactone and the acyl chain, which are further metabolized by *Variovorax paradoxus* (Leadbetter & Greenberg, 2000) and a *Ralstonia* isolate (Lin *et al*., 2003).

In a previous study, we isolated several AHL-utilizing *Arthrobacter* strains from soil using *N*-3-oxo-hexanoyl-L-homoserine lactone (OHHL) as the sole carbon source in minimal medium (Park *et al*., 2003). In the present study, however, we screened a more diverse range of AHL-utilizing bacteria on the basis of their ability to utilize non-substituted hexanoyl-L-homoserine lactone (HHL) as the sole carbon source. As a result, two strains, belonging to the genus *Rhodococcus*, were found to be significantly different in their AHL-utilization ability and in their activity to degrade various AHLs. *Rhodococcus erythropolis* W2 has previously been reported to utilize and degrade various AHLs through both oxido-reductase and AHL-acylase activites (Uroz *et al*., 2003, 2005). In contrast, the present study shows that AHLases from two *Rhodococcus* strains, LS31 and PI33, are involved in the degradation of AHL.

Materials and methods

Bacterial strains and culture media

The AHL-degrading bacteria were screened on a modified minimal medium containing HHL as the carbon source (Park *et al*., 2003). The isolated bacteria were cultivated in a nutrient broth (NB) or Luria–Bertani (LB) medium at 30 °C. *Chromobacterium violaceum* CV026 (Latifi *et al*., 1995) and *Agrobacterium tumefaciens* NT1 (pDCI41E33) (Cook *et al*., 1997), the reporter strains for the bioassay, were cultured in LB and the defined minimal medium (Zhang *et al*., 1993), respectively. The bioassay plates, using *C. violaceum* CV026 and *A. tumefaciens* NT1 (pDCI41E33), were prepared as previously described (Park *et al*., 2003). To measure the amount of residual AHLs, HHL was dispensed into wells cut into the *C. violaceum* CV026-overlaid bioassay plate, while the other AHLs except HHL were dispensed into wells cut into the *A. tumefaciens* NT1 (pDCI41E33)-overlaid bioassay plate. For the coculture, soft-rot causative *Erwinia carotovora* N98 was cultivated in LB medium at 30 °C.

Screening and isolation

Fifty milligrams of the collected soil samples were added to 2 mL of the minimal medium without a carbon source, and the mixture was vigorously vortexed. After submerging soil particles, the supernatant was inoculated (5% v/v) with 0.2 mL of the minimal medium containing 2 mM HHL and the mixture was incubated at 30 and 37 °C. After 3 days of cultivation, the same 5% (v/v) transfer was made three times to 0.2 mL of the minimal medium. After the final cultivation, the bacteria were isolated as single colonies on an NB agar, and the different types of colony on NB agar were then repeatedly cultivated in the minimal medium with and without HHL. The AHL-utilization of the isolated bacteria was also tested using 2 mM OHHL and *N*-decanoyl-L-homoserine lactone (DHL) as a carbon source. After 48 h of culture, the optical densities and residual contents of HHL, OHHL and DHL were measured using a spectrophotometer and bioassay, respectively.

Strain identification

The 16S rRNA genes of the isolated clones were PCR amplified from the colonies using the primers 9F (5′-GAGTTTGATCCTGGCTCAG), 452R (5′-GGTATTAACT TACTGCCC), and 1542R (5′-AGAAAGGAGGTGATC CAGCC). The PCR conditions were denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 45 °C for 30 s, and 72 °C for 60 s. The PCR product was then sequenced using an ABI3700 automatic sequencer (Applied Biosystems). The sequence identification was performed using the BLATAN facility of the National Center for Biotechnology Information and the sequence match facility of the Ribosomal Database Project (Maidak *et al*., 1999).

Growth experiments

The growth with HHL as the sole carbon source was determined in the minimal medium containing 2 mM HHL, by measuring the optical density at 600 nm. To measure the residual HHL content in the culture, the culture supernatant (0.1 mL) was collected and filtered. An appropriately diluted culture supernatant was then loaded into a well cut into a *C. violaceum* CV026 overlaid plate, and the diameter of the purple zone was measured after incubation at 30 °C for 24 h. The relative amount of HHL was calculated using the following regression equation, HHL (pmol) = $0.8217e^{1.734x}$, where x represents the diameter of the purple zone.

Bioassay of AHL-degrading activity

The AHL substrates, *N*-octanoyl-L-homoserine lactone (OHL) and *N*-3-oxododecanoyl-L-homoserine lactone (OdDHL), were purchased from Quorum Sciences Inc. and Fluka, while HHL, OHHL and DHL were all synthesized at our laboratory, as described previously (Zhang *et al*., 1993). For the whole cell assay, overnight cultures of the isolates were washed and resuspended with 100 mM potassium phosphate buffer (pH 7.5). Then, 50 μL of the cell resuspension ($OD_{600\,nm} = 1.0$) and an equal volume of AHLs (final concentration, 20 μM) were mixed, and the mixture was incubated at 30 °C with gentle agitation. After boiling at 95 °C for 5 min to stop the reaction, the reaction mixture was diluted to an appropriate concentration and loaded into

a well in a reporter strain overlaid plate. Thereafter, 0.5 pmol of OHHL, 25 pmol of OHL, and 100 pmol each of DHL and OdDHL were separately loaded on *A. tumefaciens* NT1 (pDCI41E33)-overlaid minimal agar plates, and 100 pmol of HHL was loaded on *C. violaceum* CV026-overlaid LB plates. The residual AHL content was then calculated using a regression equation, based on the color zone size and known amounts of AHLs.

Coculture of *E. carotovora* and two *Rhodococcus* strains

For the coculture experiments, the *E. carotovora* N98 was cultivated together with either *Rhodococcus* sp. strain LS31 or strain PI 33 in LB medium to an initial cell density ratio of 1 or 3. The *E. carotovora* N98 and two *Rhodococcus* strains were inoculated into LB medium to an initial cell density of $1–2 \times 10^6$ CFU mL^{-1}, respectively. After 12 h of incubation, samples were withdrawn to determine the OHHL content and pectate lyase activity. To quantify the OHHL synthesized, the culture supernatant was filtered and loaded onto *A. tumefaciens* NT1 (pDCI41E33)-overlaid bioassay agar plates. The extracellular pectate lyase activity was determined as described previously (Laurent *et al.*, 2000). One unit of enzyme activity was defined as 0.01 absorbance increase at 235 nm min^{-1} mL^{-1} of supernatant.

Liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI/MS) analysis

The reaction for AHL degradation was carried out in 200 μL of 10 mM potassium phosphate buffer (pH 7.5) containing 2 mM HHL and the cell suspension ($OD_{600\,nm} = 5.0$). The reaction mixture was incubated at 30 °C with gentle shaking for 3 h. After centrifugation of the reaction mixture, the supernatants were filtered and applied to YMC-Hydrosphere C_{18} reverse phase column (2.0×50 mm). Mass spectra were obtained via liquid chromatographic introduction into a Finnigan LCQ Advantage MAX ion trap mass spectrometry, equipped with a Finnigan Surveyor Modular HPLC (Thermo Electron Co.). Mobile phases A and B were water and acetonitrile containing 0.1% formic acid, respectively. Elution was conducted according to the following profile at a flow rate of 0.2 mL min^{-1}: 0–20 min for 5–60% mobile phase B and 20–35 min for 100% mobile phase B. All the experiments were carried out under automatic gain control condition.

Results

Isolation and identification of AHL-utilizing bacteria

To isolate AHL-degrading bacteria, soil, leaf, and moss samples were cultivated in a minimal medium containing HHL as the sole carbon source. After three rounds of enrichment culture, the cell suspension was spread on NB agar, and different types of colony were isolated. Finally, eight representative strains were selected on the basis of growth and the residual content of HHL in the culture medium. The isolated strains were identified mainly as the members of the genera *Pseudomonas* (PA12, PA23), *Acinetobacter* (PA21, PI42), *Ralstonia* (LS21), *Arthrobacter* (PI51), and *Rhodococcus* (PI33, LS31), based on the analysis of the 16S rRNA gene sequences (Table 1). Although the genera *Pseudomonas, Ralstonia*, and *Rhodococcus* have already been reported to utilize AHL as a carbon source, and AHL-degrading activity was found in *Acinetobacter* (Kang *et al.*, 2004), this is the first report of an AHL-utilizing *Acinetobacter*. The eight isolates were tested for their utilization of OHHL and DHL, as well as HHL (Table 1). Several strains, including PI33, PI42, and LS21, exhibited higher utilization ability toward unsubstituted AHLs, such as HHL and DHL,

Table 1. Representative AHL-degrading bacteria isolated from soil samples

			Residual AHL assay*		
Strain	Colony morphology	Identification (16S rRNA gene sequence homology)	HHL	OHHL	DHL
PA12	White	*Pseudomonas aeruginosa* (99%)	+	ND	ND
PA23	White	*Pseudomonas citronellolis* ATCC 13674^T (99%)	+++	ND	ND
PA21	White	*Acinetobacter calcoaceticus* ATCC 23055^T (97%)	++	ND	ND
PI42	White	*Acinetobacter baumannii* ATCC 19606^T (100%)	+	–	+++
LS21	Ivory, viscous	*Ralstonia basilensis* DSM 11853 (99%)	+	–	+++
PI51	Yellow	*Arthrobacter histidinolovorans* DSM 20115 (98%)	+++	+++	+++
LS31	White, viscous	*Rhodococcus erythropolis* DSM 43066^T (99%)	+++	++	+++
PI33	Pink	*Rhodococcus ruber* DSM43338^T (99%)	+++	–	+++

*The amount of remaining AHLs was measured using bioassay after cultivation in a minimal medium containing 2 mM AHLs for 48 h. The number of plus symbol relates to the amount of AHLs degraded: +, 20–50% degradation of initial AHLs; ++, 50–80%; +++, 80–100%.
ND, not determined; AHL, *N*-acylhomoserine lactone; HHL, hexanoyl-L-homoserine lactone; OHHL, *N*-3-oxo-hexanoyl-L-homoserine lactone; DHL, *N*-decanoyl-L-homoserine lactone.

than the 3-oxo substitute OHHL. In contrast, however, strains PI51 and LS31, identified as *Arthrobacter* and *Rhodococcus*, respectively, grew well on all the AHLs tested, and no residual AHL was detected after 48 h of cultivation. Interestingly, even though the two strains, LS31 and PI33, shared 99.2% and 99.4% sequence identity with the 16S rRNA gene sequence of *R. erythropolis* and *Rhodococcus ruber*, respectively, and they were from the same genus *Rhodococcus*, their ability to utilize OHHL was significantly different. Therefore, in order to compare and analyze different AHL-degrading capacities within the same genus, the two *Rhodococcus* strains LS31 and PI33 were selected for the following study.

Growth of *Rhodococcus* sp. strains LS31 and PI33

The growth of *Rhodococcus* sp. strain LS31 and strain PI33 was examined in a minimal medium containing 2 mM HHL as the sole carbon source, and the residual HHL contents in the medium were assessed during growth. *Rhodococcus* sp. strain PI33 utilized the HHL much more efficiently than the LS31, with specific growth rates of 0.277 and 0.065 h^{-1}, respectively (Fig. 1). Consistent with the growth rate data, strain LS31 exhausted the HHL more slowly in proportion to its growth during 40 h of cultivation (Fig. 1a), whereas the strain PI33 consumed most of the HHL after 10 h of cultivation (Fig. 1b). No growth of the strains LS31 and PI33 was observed in an HHL-depleted minimal medium (data not shown). As the pH of the culture medium was kept at about 6.0 during growth (Fig. 1), the decrease of HHL was not due to chemical hydrolysis in an alkaline pH.

Substrate specificity of *Rhodococcus* sp. strains LS31 and PI33

To compare the substrate specificity of *Rhodococcus* sp. strain LS31 and PI33, their degrading activities of various AHLs were determined using a whole cell assay. Thus, the strains LS31 and PI33 were grown in NB, and whole cells were then exposed to different AHLs. *Rhodococcus* sp. strain LS31 effectively degraded the AHLs tested, regardless of their length and acyl side chain substitution, whereas boiled *Rhodococcus* sp. LS31 failed to degrade HHL (Fig. 2a). As seen in the figure, the strain PI33 exhibited a high degrading activity towards OHL and DHL, similar to *Rhodococcus* sp. strain LS31. However, the activity towards 3-oxo-substituted AHLs, OHHL and OdDHL, was less than that of the strain LS31. In particular, the strain LS31 eliminated most of the OHHL within 30 min, whereas the stain PI33 showed significantly lower activity towards OHHL, suggesting that the AHL-degrading enzymes of the two *Rhodococcus* strains have quite different AHL substrate preference.

Effect of *Rhodococcus* sp. strains LS31 and PI33 on quorum sensing of *E. carotovora*

The two *Rhodococcus* strains exhibited different degradation properties with regards to their substrate preferences as well as degradation kinetics. Thus, to evaluate whether these AHL-degradation properties had any effect on the potential of the strain to interfere with quorum sensing regulated functions, strains LS31 and PI33 were separately cocultures with *E. carotovora,* which makes OHHL and responds to it by regulating the expression of exoenzymes such as pectate lyase (Park *et al*., 2003). Thus, each *Rhodococcus* strain was mixed with *E. carotovora* at different CFU ratios and co-cultured in LB medium at 30 °C. The growth of *E. carotovora* ($\mu = 1.0$–1.2) was unaffected by the initial inoculum size of the *Rhodococcus* strains during cultivation with strains LS31 and PI33 at different CFU ratios (data not shown). On the other hand, the strain LS31 efficiently degraded OHHL produced by *E. carotovora*, resulting in 88% and 89% reduction of OHHL concentration and the pectate lyase activity, respectively, even with an equal initial CFU ratio

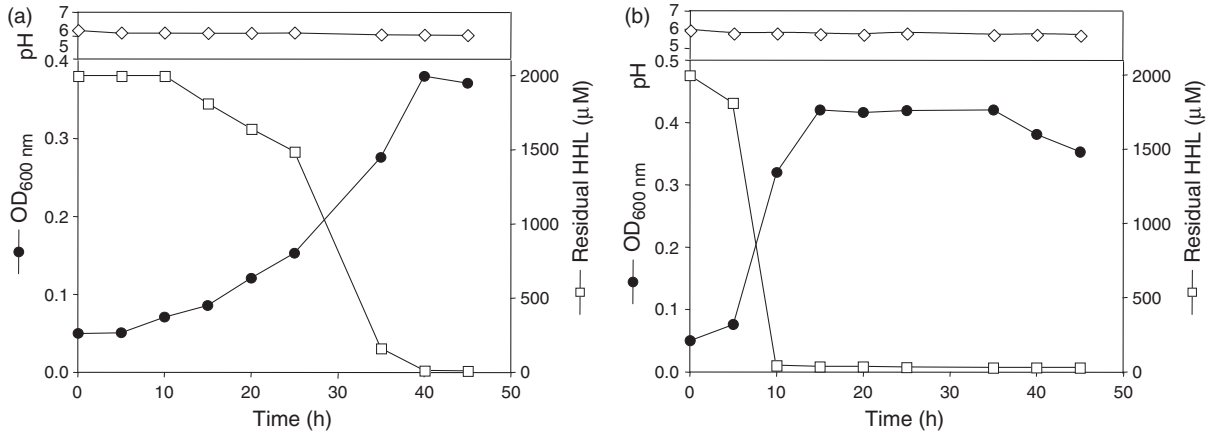

Fig. 1. Growth and residual HHL for *Rhodococcus* sp. strains LS31 (a) and PI33 (b) in minimal medium. The residual activity means the amount of remaining AHL in *in vitro* assay. ●, absorbance at 600 nm; □, residual HHL; ◇, pH of culture.

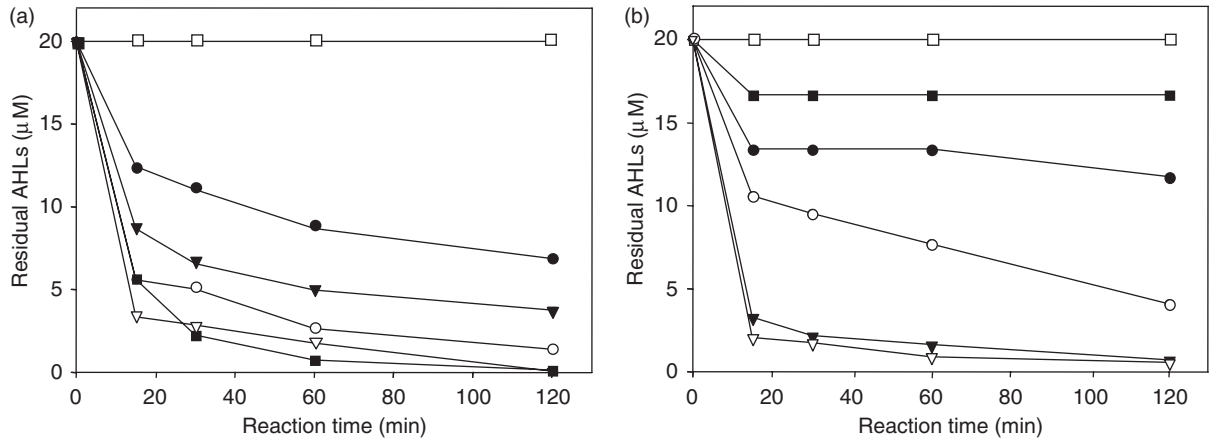

Fig. 2. Substrate specificity of *Rhodococcus* sp. strains LS31 (a) and strain PI33 (b). The residual activity means the amount of remaining AHL in *in vitro* assay. ●, HHL; ■, OHHL; ▽, OHL; ▼, DHL; ○, OdDHL; □, HHL with boiled culture samples.

Table 2. Effect of *Rhodococcus* sp. strains LS31 and PI33 on OHHL accumulation and pectate lyase activity of *Erwinia carotovora*

Culture	Ratio*	OHHL (μM)†	Pectate lyase activity (U mL^{-1})†
E. carotovora	–	1.2 ± 0.24	550.0 ± 12.0
Ec: *Rhodococcus* sp. strain LS31	1 : 1	0.1 ± 0.01	50.0 ± 7.0
Ec: *Rhodococcus* sp. strain LS31	1 : 3	0.1 ± 0.02	50.2 ± 3.0
Ec: *Rhodococcus* sp. strain PI33	1 : 1	0.5 ± 0.07	364.5 ± 10.1
Ec: *Rhodococcus* sp. strain PI33	1 : 3	0.3 ± 0.08	260.2 ± 12.1

*The initial cell density was denoted by the ratio of *E. carotovora* to *Rhodococcus* sp. strains LS31 and PI33.

†The values present the mean values and standard deviations of three independent experiments. The values were obtained after 12 h of coculture.

Ec, *Erwinia carotovora*; OHHL, *N*-3-oxo-hexanoyl-L-homoserine lactone.

(Table 2). Cocultivation with strain PI33 resulted in about 50% reduction of the amount of OHHL and pectate lyase activity, compared with that of *E. carotovora* only, even when the initial CFU ratio was increased to 1 : 3. These results suggest that high OHHL-degrading activity of *Rhodococcus* sp. strain LS31 is able to control the virulence of *E. carotovora* more successfully than *Rhodococcus* sp. strain PI33.

AHL-degrading mechanism by *Rhodococcus* sp. strains LS31 and PI33

The different AHL-degrading activity of the two *Rhodococcus* sp. strains LS31 and PI33 prompted us to examine the mechanism of AHL-degradation. The strains LS31 and PI33 cultivated in NB medium were exposed to HHL for 3 h, and the reaction product was then identified by LC-ESI/MS. As shown in Fig. 3a, the reaction of strains LS31 and PI33 with HHL generated a single product with a retention time of 8.4 min on mass chromatogram. The rate of the production was higher with stain LS31 than with strain PI33, which is consistent with the lower HHL-degrading activity of strain PI33 than the strain LS31. The ESI-MS analysis of the product revealed a quasimolecule M+H ion at *m*/*z* 218, suggesting that the enzymatic reaction of HHL (M+H ion *m*/*z* 200) led to the opening of homoserine lactone by the addition of water and resulted in mass increase of 18 (Fig. 3b). The result led us to postulate that the addition of water was caused by AHLases produced by LS31 and PI33 strains.

Discussion

The biological degradation of quorum-sensing signal molecules is an important way of interrupting the quorum sensing. In this study, using non-substituted HHL as the sole carbon source, diverse AHL-utilizing bacteria were obtained, including the genera *Acinetobacter, Pseudomonas, Ralstonia,* and *Rhodococcus*, as well as *Arthrobacter* strain. Interestingly, in an enrichment culture based on HHL, most of the strains isolated showed a much lower utilization ability of 3-oxo substituted HHL than non-substituted HHL and DHL. In particular, two strains LS31 and PI33 which belong to the same genus, *Rhodococcus*, exhibited significantly different activities towards 3-oxo substituted AHLs (Table 1).

Strain PI33 eliminated the HHL more rapidly than strain LS31 during growth on the HHL-containing minimal medium (Fig. 1), although the AHL-degrading activity of strain LS31 was higher than that of strain PI33, when they were cultivated in NB medium (Fig. 2). In contrast to strain LS31, the degrading activity of HHL, rather than the other AHLs, increased when strain PI33 was cultivated on NB medium in the presence of 0.3 mM HHL (data not shown), suggesting that strain PI33 has at least one inducible enzyme involved in the degradation of HHL. In the case of strain LS31, the relative degradation activity of OHHL was lower than those of HHL and DHL, when cultivated in a minimal medium

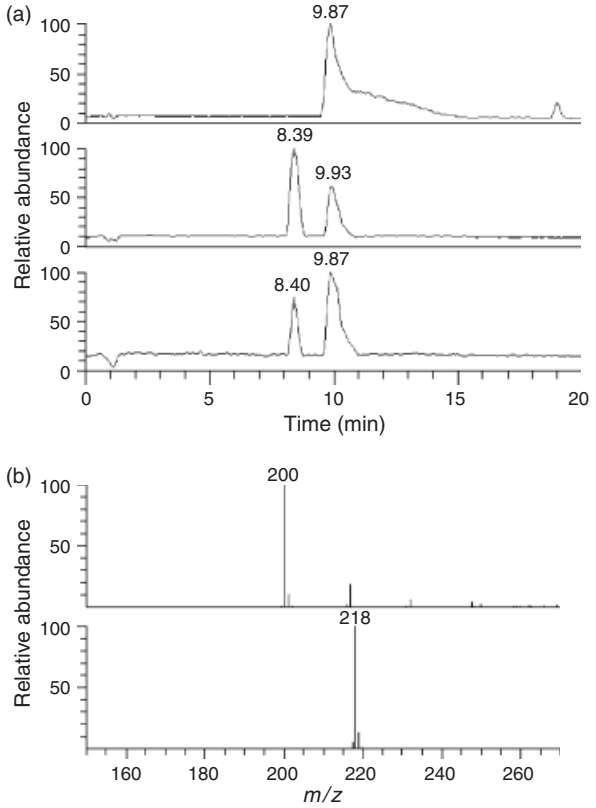

Fig. 3. LC-ESI/MS chromatogram (a) and MS spectrum (b) of HHL degradation product. (a) *Rhodococcus* sp. strains LS31 (middle) and PI33 (bottom) were reacted with 2 mM HHL for 3 h. As a standard, HHL (top) was used. (b) The peak of LC/ESI-MS chromatogram at 9.9 (top) and 8.4 (bottom) was analyzed by ESI-MS.

(Table 1); however, OHHL degradation was greater than that of HHL and DHL in NB medium (Fig. 2a). This implies that AHL-degrading enzymes or their genes are either induced or repressed, depending on the media. Therefore, the *Rhodococci* appears to have unique mechanisms, showing different expression dynamics for degradation and utilization of AHLs.

Recently, an AHL-degrading *R. erythropolis* W2, which showed relatively higher AHL-degrading activity against 3-oxo-substituent than unsubstituted AHLs, was isolated from a tobacco rhizosphere (Uroz *et al.*, 2003, 2005). However, strain LS31 was able to degrade various AHLs with different lengths and acyl side-chain substitutions (Fig. 2a), although it is same species as *R. erythropolis* W2. Furthermore, AHLases from both strains LS31 and PI33 were involved in AHL degradation (Fig. 3), whereas *R. erythropolis* W2 was capable of modifying and degrading AHL through both oxidoreductase and acylase activities. These results indicate that *Rhodococcus* strains have different AHL-degrading properties and metabolic pathways to utilize AHLs, even though they belong to the same genus.

The identification of AHL-degrading activity in two different *Rhodococcus* strains suggests that many *Rhodococcus* species may express AHL-degrading enzymes. Therefore, to explore this possibility, six different *Rhodococcus* type strains (*Rhodococcus coprophilus* KCTC 9804, *Rhodococcus equi* KCTC 1298, *Rhodococcus erythropolis* KCTC 3483, *Rhodococcus jostii* KCTC 19938, *Rhodococcus opacus* KCTC 9811, and *Rhodococcus rhodochrous* KCTC 9086) were obtained from Korean Collection for Type Cultures (KCTC) and tested for their AHL-degrading activities. All the *Rhodococcus* strains tested showed AHL-degrading activities in a whole cell assay (data not shown), indicating that AHL-degrading enzymes are probably universally present among the genus *Rhodococcus,* irrespective of the nature of the isolates. The genus *Rhodococcus* is a member of Gram-positive nocardioform actinomycete group and exhibits a remarkable ability to utilize a wide variety of natural organic and xenobiotic compounds. Therefore, the AHL-degrading activity of *Rhodococcus* species described herein expands the list of diverse metabolic traits exhibited by the members of this bacterial species.

It has been proposed that bacterial strains with AHL-degrading enzymes may compete with Gram-negative bacteria in the natural ecosystem. Indeed, coculture of *Rhodococcus* spp. with *E. carotovora* showed that AHL-degrading bacteria could interfere with the quorum sensing of Gram-negative bacteria (Table 2), although the actual roles of the AHL-degrading enzymes in *Rhodococcus* sp. and in the natural environment remain unclear. Nevertheless, the presence of AHL-degrading enzyme in many *Rhodococcus* species suggests another role, in addition to competition against AHL-producing quorum sensing bacteria. Thus, more studies are needed to characterize the properties of proteins involved in AHL signal degradation and elucidate physiological function of the AHL-degrading enzyme in *Rhodococcus* sp. strains LS31 and PI33.

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