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Metabolism of Acyl-Homoserine Lactone Quorum-Sensing Signals by *Variovorax paradoxus*

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Acyl-homoserine lactones (acyl-HSLs) serve as dedicated cell-to-cell signaling molecules in many species of the class *Proteobacteria*. We have addressed the question of whether these compounds can be degraded biologically. A motile, rod-shaped bacterium was isolated from soil based upon its ability to utilize *N*-(3-oxohexanoyl)-L-homoserine lactone as the sole source of energy and nitrogen. The bacterium was classified as a strain of *Variovorax paradoxus*. The *V. paradoxus* isolate was capable of growth on all of the acyl-HSLs tested. The molar growth yields correlated with the length of the acyl group. HSL, a product of acyl-HSL metabolism, was used as a nitrogen source, but not as an energy source. Cleavage and partial mineralization of the HSL ring were demonstrated by using radiolabeled substrate. This study indicates that some strains of *V. paradoxus* degrade and grow on acyl-HSL signals as the sole energy and nitrogen sources. This study provides clues about the metabolic pathway of acyl-HSL degradation by *V. paradoxus*.

A diversity of bacterial species self-regulate expression of specific sets of genes in response to their own population density, a phenomenon that has become known as quorum sensing (for reviews, see references 9, 27, and 33). Many members of the class *Proteobacteria* have quorum-sensing systems that rely on acyl-homoserine lactone (acyl-HSL) signals. The nature of the acyl side chain of the signal molecule depends upon the quorum-sensing system. A diversity of acyl-HSL structures have been elucidated. Signal specificity depends on the length of and the substitutions in the acyl side chain (Fig. 1). Acyl-HSLs are dedicated signaling molecules with no other known function, and a specific enzyme is required for their synthesis (13, 20, 22, 30). These signal molecules reach concentrations on the order of 10 μM in laboratory cultures of quorum-sensing bacteria (5, 24, 26).

The available evidence is consistent with the idea that bacteria which synthesize acyl-HSLs do not degrade them, and acyl-HSLs are chemically stable at neutral or acidic pH in aqueous solutions (29). However, the HSL ring is subject to alkaline hydrolysis (32). The potential for biological decomposition of these signals is intriguing for several reasons. Other bacteria sharing the same local environment as quorum-sensing bacteria could conceivably gain a competitive advantage by degrading acyl-HSL signals. Enzymes that degrade acyl-HSLs might have commercial value as modulators of cell-to-cell signaling. Since acyl-HSLs are stable under slightly acidic conditions, biological degradation could play an important role in maintaining these signals at low environmental concentrations.

A recent report shows that acyl-HSL signaling molecules can be biologically inactivated by specific soil bacteria (4). A gene encoding this degradative ability was cloned from a *Bacillus* isolate. The purified gene product showed acyl-HSL-inactivating ability. It was not clear how the gene product served to inactivate acyl-HSLs or whether the *Bacillus* could use acyl-HSLs as nutrients for growth. To initiate our investigations into the biological degradation of acyl-HSL molecules, we have used enrichment and isolation techniques to obtain a pure culture of a bacterium capable of utilizing these signals as the sole source of energy and nitrogen. This is our initial description of that bacterium and its acyl-HSL-degrading capabilities.

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MATERIALS AND METHODS

Bacterial strains, media, and culture conditions. The bacterial strains used were *Variovorax paradoxus* VAI-C (isolation described below), *V. paradoxus* ATCC 17713, and, for biological production of radioactive *N*-butanoyl-HSL (C4-HSL), *Escherichia coli* XL1-Blue containing pRHL1 (21). For growth of *E. coli*, we used Luria-Bertani (28) broth or agar amended as indicated. For enrichment, isolation, and growth experiments with *V. paradoxus*, we used a defined medium. The composition of the medium (per liter) was 1 g of NaCl, 0.5 g of KCl, 0.4 g of $MgCl_2 \cdot 6H_2O$, 0.3 g of NH_4Cl (unless otherwise specified), 0.1 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of KH_2PO_4, 0.15 g of Na_2SO_2, and 1 g of 2-(*N*-morpholino)-ethanesulfonic acid (MES). Trace elements and a selenate-tungstate mixture (18) were added, and the pH was adjusted to 5.5 with 1 M NaOH. This basal medium was autoclaved, and, after cooling, vitamins were added unless otherwise noted. The final vitamin composition per liter of medium was 100 μg of riboflavin and 1 mg of L-ascorbic acid, biotin, DL-calcium-panthothenate, folic acid, niacinamide, nicotinic acid, *p*-aminobenzoic acid, pyridoxal-HCl, thiamine-HCl, lipoic acid, and cyanocobalamin. For cultivation on solid media, agarose (Gibco Ultrapure) was incorporated at a final concentration of 1.0%. Growth substrates were added to the autoclaved, vitamin-amended medium as indicated.

For routine maintenance of *V. paradoxus* we used the medium described above supplemented with 5 g (wt/vol) of Difco yeast extract $\cdot$ liter^{-1} as an energy and nitrogen source. Stock solutions of acyl-HSLs were used at 5 mg $\cdot$ ml^{-1} (except for *N*-3-oxohexanoyl-L-HSL [3OC6-HSL], which was used at 50 mg $\cdot$ ml^{-1}) in ethyl acetate acidified with glacial acetic acid (0.1% [vol/vol]). The stock solutions were stored at −20°C. For liquid media, the solutions of acyl-HSLs were dispensed into sterile tubes, the ethyl acetate was removed by evaporation under a stream of nitrogen gas, and sterile medium was added to the remaining acyl-HSL. The acyl-HSL-containing media were used immediately after preparation. Acyl-HSLs with carbon chain lengths of >8 did not fully dissolve in the medium at concentrations of >100 μM. Cells were grown in 3 or 5 ml of medium in 13- or 18-mm-diameter tubes, respectively, with shaking at 30°C unless otherwise noted. For agarose plates, the ethyl acetate solutions were spread on the surface of the agarose medium, and the plates were used shortly after the ethyl acetate evaporated. Acyl-HSL molecules are stable for weeks under the conditions of low pH in our defined medium. At higher pH values (ca. 8.0), the half-life of an acyl-HSL can be <3 h (29; A. Eberhard, personal communication).

Enrichment and isolation procedures. Turf soil was collected in September 1998 at the University of Iowa. The soil was disrupted with a metal spatula until all particles were finely dispersed, and the remaining large particles were removed. One hundred milligrams of the soil preparation was added to 3 ml of the basal medium containing 3OC6-HSL as the sole source of nitrogen and energy (500 μg $\cdot$ ml^{-1}). Vitamins were not added to the enrichment medium. After 48 h, a 5% (vol/vol) transfer was made to fresh enrichment medium, and after an additional 48 h, a second transfer was made. After a further 48-h incubation, cells

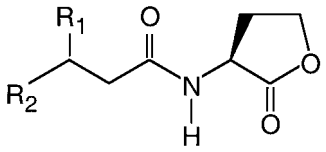

FIG. 1. Generalized structure of acyl-HSLs produced by quorum-sensing bacteria: R_1, -H, -OH, or =O; R_2, $-CH_3$, $-(CH_2)_{2-10}CH_3$ or $-(CH_2)_3CH{=}CH(CH_2)_5CH_3$.

in the third transfer tube were streaked on a plate of 3OC6-HSL-containing agarose medium.

Growth studies. We constructed a 3OC6-HSL consumption curve by analyzing duplicate 10-μl samples of culture fluid collected during growth. Duplicate 1-ml samples were collected after the culture had entered the stationary phase. The analysis was by means of a 3OC6-HSL bioassay as described elsewhere (23).

Molar growth yields with acyl-HSLs as energy sources were determined in NH_4Cl-replete medium containing the indicated acyl-HSL at a final concentration of 0.5, 0.75, 1.0, or 1.5 mM. Growth yields with nitrogen sources other than NH_4Cl were determined in a medium containing 20 mM sodium succinate as the energy source. The nitrogen sources used in place of NH_4Cl were HSL (at concentrations of 0 to 10 mM), homoserine (at concentrations of 0 to 10 mM), or 3OC6-HSL (at concentrations of 0 to 1 mM). A factor for converting optical density to cell dry mass was constructed by using cells grown in a medium containing succinate as the energy source and NH_4Cl as the nitrogen source, washed with 50 mM ammonium acetate buffer (pH 5.5), and then dried to a constant weight. Experiments were done at least twice.

Metabolism of radiolabeled C4-HSL. We prepared C4-L-[1-^{14}C]HSL for radiotracer experiments by modification of a previously described procedure (12). *E. coli* XL1-Blue cells containing the C4-HSL synthase expression vector pRHLI were grown in 50 ml of Luria-Bertani broth containing ampicillin (100 $\mu g \cdot ml^{-1}$). Isopropyl-β-thiogalactoside (1 mM) was added after 2 h at 37°C. Cells were harvested by centrifugation when the culture reached an optical density of 0.7 at 600 nm. The cells were suspended in 2 ml of phosphate-buffered saline (28) containing 10 mM glucose in a 15-ml conical tube. After 10 min at 37°C with shaking, we added 10 μCi of L-[1-^{14}C]methionine (55 $mCi \cdot mmol^{-1}$; American Radiolabeled Chemicals, Inc., St. Louis, Mo.) and incubated the cell suspension for an additional 4 h. The cells were then removed by centrifugation, and the C4-HSL was extracted from the remaining culture fluid with 2 equal volumes of acidified ethyl acetate. The ethyl acetate evaporated, and the residue was dissolved in 200 μl of 20% methanol in water. The C4-HSL in the methanol-water was purified by reversed-phase high-performance liquid chromatography (29). The purified, radioactive C4-HSL was dried and stored at −20°C. Radioactivity was measured with a liquid scintillation counter and was quench corrected by using an internal standard.

The fate of radioactive C4-HSL during growth of *V. paradoxus* VAI-C was assessed in the following manner: *V. paradoxus* was cultured in 5 ml of medium in 25-ml butyl rubber-stoppered tubes containing sufficient concentrations of oxygen for aerobic growth. Radioactive C4-HSL (10 μmol, 32 $\mu Ci \cdot mmol^{-1}$) was added to each reaction vessel (as described above). Immediately after inoculation, the amount of radioactivity in the culture broth was measured. After cultures had reached the stationary phase, the headspace above each culture was flushed with N_2 for 60 min into two CO_2 traps connected in tandem (1). The radioactivity in the second vial ranged from 0.1 to 1.0% of that trapped in the first. Culture fluid was then removed, and after centrifugation, the clarified culture fluid was extracted with 8 equal volumes of acidified ethyl acetate. Radioactivity in both the extract and the culture fluid after extraction was measured. The cell pellet was washed three times with 10 mM MES (pH 5.5). The radioactivity present in the washed cells was determined after boiling for 2 min in 100 μl of 1 M NaOH.

Nucleotide sequence analysis of the 16S rDNA. The nucleotide sequence of a PCR-amplified fragment of the 16S ribosomal DNA (rDNA) of our bacterial isolate was determined by previously described procedures (18). Genomic DNA was isolated with a QIAamp tissue kit (QIAGEN, Inc., Valencia, Calif.). We used the Expand Long Template PCR system (Boehringer-Mannheim) to amplify 16S rDNA with about 50 ng of bacterial DNA as the template with previously described 27-forward and 1494-reverse primers (17). The PCR product was purified and ligated into pCRII by using a TOPO TA cloning kit (Invitrogen, San Diego, Calif.). DNA sequencing was by routine automated methods at the University of Iowa DNA Core Facility. The sequencing primers were the standard M13 forward and M13 reverse primers and primers previously designed to target internal regions of the 16S rRNA genes of most bacteria (17). For sequence analysis, we used ARB software (www.mikro.biologie.tu-muenchen.de/pub/ARB/linux/).

Other analyses. Phase-contrast microscopy and epifluorescence microscopy were performed with an Olympus BH2 microscope. Acridine orange staining and confocal-epifluorescence microscopy were performed as described elsewhere (2, 6). To demonstrate that HSL was released as a product of acyl-HSL degradation during culture growth, growth supernatants were analyzed at the University of Iowa College of Medicine Molecular Analysis Facility with a Beckman 6300 high-performance ion-exchange analyzer operated according to the manufacturer's specifications. The bioassays used to screen for production of 3OC6-HSL, C4-HSL, and related molecules by *V. paradoxus* have been described previously (23, 25).

Nucleotide sequence accession number. The 16S rDNA sequence has been assigned GenBank accession no. AF250030. All other rDNA sequences were from the ARB database or from GenBank.

RESULTS

Enrichment and isolation of acyl-HSL-degrading bacteria. Enrichment was in vitamin-free basal medium containing 3OC6-HSL. Enrichment tubes were inoculated with soil, and growth was evident as turbidity within 48 h. No obvious turbidity was observed in the absence of 3OC6-HSL. After two transfers in the 3OC6-HSL-containing medium, a complex microbial community including a variety of bacterial and eukaryotic microbes was observed by phase-contrast microscopy. There was an obvious biofilm near the air-liquid interface. The biofilm was disrupted by vortexing the culture for 30 s, and then a sample of the culture was streaked on a plate of vitamin-free agarose medium containing 3OC6-HSL. Three colony types arose over a period of a week. Pure cultures of each colony type were obtained by repeated streaking. The three isolates obtained were designated VAI-A (a dimorphic rod-coccus), VAI-B (a highly motile spirillum), and VAI-C (a weakly motile rod). These isolates were screened for growth on 3OC6-HSL in the liquid medium used for the enrichments. The pure VAI-A and VAI-C cultures grew on 3OC6-HSL, and VAI-C was chosen for further study.

Phylogenetic analysis of VAI-C. A nearly complete sequence for the 16S rDNA was obtained. The sequence corresponds to *E. coli* 16S rRNA nucleotide positions 28 to 1489. Web-based similarity searches against the GenBank and Ribosomal Database Project databases suggested that VAI-C belonged to the subclass β-*Proteobacteria*, clustering with the family *Comamonadaceae*. The 16S rDNA of VAI-C shared 99.3 to 99.8% sequence identity with the 16S rDNA of three strains of *V.* (formerly *Alcaligenes*) *paradoxus*. A further phylogenetic analysis supported the conclusion that VAI-C is a strain of *V. paradoxus*. By any of the FastDNAML maximum-likelihood, maximum-parsimony, and Desoete distance treeing algorithms, VAI-C clustered tightly with the other *V. paradoxus* strains (Fig. 2). Thus, we consider our isolate to be a strain of *V. paradoxus*. An additional confirmation of this assignment was ob-

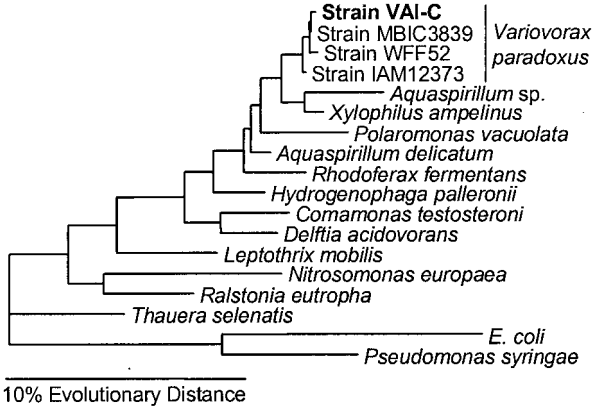

FIG. 2. 16S rRNA-based evolutionary tree showing the phylogenetic position of strain VAI-C. The horizontal bar at the bottom represents a 10% difference in evolutionary distance as determined by measuring the lengths of the horizontal lines connecting the species. *Pseudomonas syringae* and *E. coli* were used as outgroups.

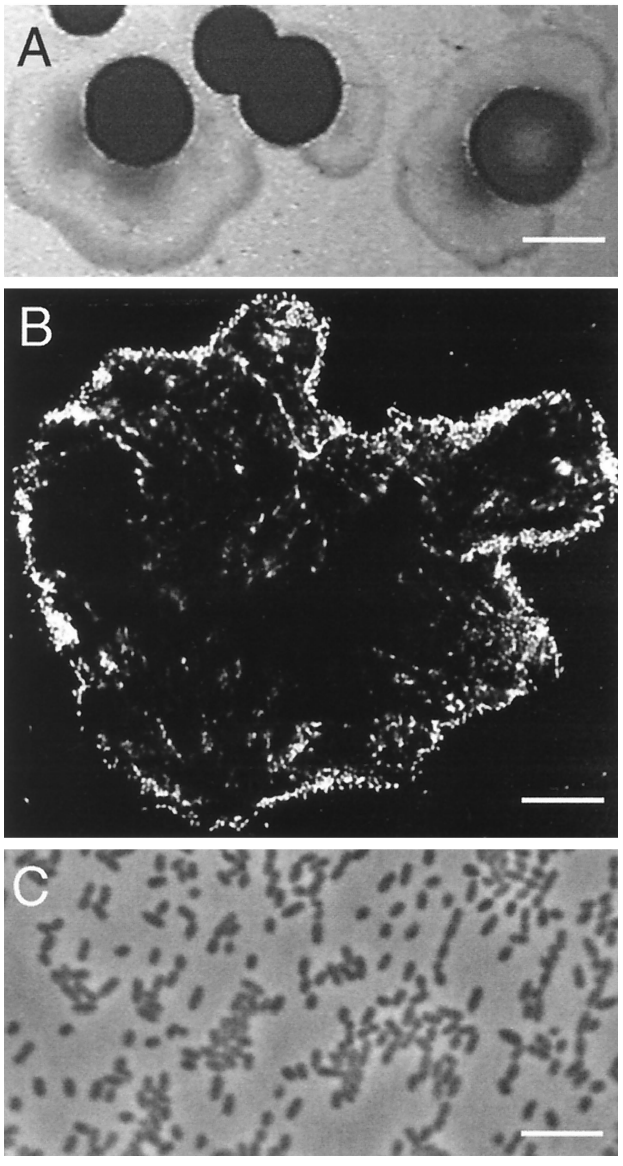

FIG. 3. Colonial and cell morphology of *V. paradoxus* VAI-C. (A) Photograph of colonies grown on yeast extract-agarose plates. (B) Confocal fluorescence micrograph of acridine orange-stained cells attached to the surface of a particle of C12-HSL. (C) Phase-contrast micrograph of 3OC6-HSL-grown cells. Bars are 4 mm (A), 17 μm (B), and 5 μm (C).

tained by demonstrating that the type strain of *V. paradoxus* (ATCC 17713) was capable of growth in 3OC6-HSL broth. Growth of this strain was not as rapid as growth of VAI-C in the test medium, irrespective of the substrate tested (data not shown).

General properties of *V. paradoxus* VAI-C. Growth of *V. paradoxus* VAI-C on 3OC6-HSL occurred at 30°C, but not at 37°C. The isolate formed spreading, yellow colonies on plates of 3OC6-HSL defined agarose medium and raised, yellow colonies with an occasional spreading edge on yeast extract agarose plates (Fig. 3A). Cells from late-logarithmic-phase 3OC6-HSL broth cultures were 1.1 by 0.7 μm in dimension (Fig. 3C). Despite the spreading of colonies on agarose plates, cells in broth were sporadically motile. A putrid aroma was produced during growth, especially on yeast extract agarose plates. As is the case for other strains of *V. paradoxus*, our isolate was capable of growth with pantothenic acid or folic acid as the sole source of energy and nitrogen (10, 11, 19).

To examine whether *V. paradoxus* VAI-C produced an acyl-HSL, we extracted culture fluid with ethyl acetate and used bioassays for C4-HSL, 3OC6-HSL, and related acyl-HSLs to screen the concentrated extracts (see Materials and Methods). We did not detect acyl-HSLs in extracts obtained from low-pH defined medium amended with 0.5% yeast extract or from medium with succinate as the carbon and energy source.

Acyl-HSLs as energy sources for growth. Pure cultures of *V. paradoxus* VAI-C grew slowly (24- to 48-h doubling times) on 3OC6-HSL in the absence of vitamins. In vitamin-supplemented medium, the doubling time improved to 18 h, with a molar growth yield of 94 g (dry weight) of cell · mol of 3OC6-HSL^{-1}. With NH_4Cl added to the medium, the doubling time improved to 3.5 h (Fig. 4); the molar growth yield with NH_4Cl was about the same as without it (as was the final pH in the culture medium [5.7 to 6.4]). The concentration of 3OC6-HSL decreased during logarithmic growth, and it was below 100 nM just after the onset of the stationary phase (Fig. 4).

Growth of *V. paradoxus* VAI-C occurred with a diversity of acyl-HSLs (Table 1). In fact, there was growth with the complete series of saturated acyl-HSLs we tested. Acyl-HSLs with acyl chains ≥8 carbons did not completely dissolve in the medium at the concentrations we used (>100 μM), so accurate growth rates on these substrates were not obtained. However, upon incubation, the insoluble acyl-HSL particles disappeared, and final culture densities could be measured. Epifluorescence microscopy of acridine orange-stained cells during early growth on C12-HSL showed acyl-HSL particles covered with *V. paradoxus* cells (Fig. 3B). Few cells were found unattached in the growth medium until the visible C12-HSL particles had disappeared.

The molar growth yields on different acyl-HSLs showed a direct correlation with the lengths of their acyl side chains (Fig. 5). This is consistent with the conclusion that the acyl group

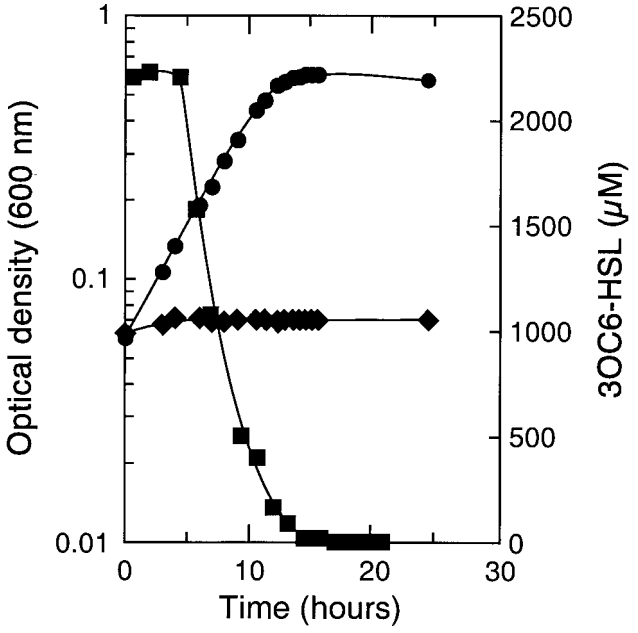

FIG. 4. Growth of *V. paradoxus* VAI-C with 3OC6-HSL as the sole energy source. Culture density in medium with (●) and without (◆) 3OC6-HSL is shown, as is the concentration of 3OC6-HSL in the 3OC6-HSL-containing culture (■).

TABLE 1. Growth of *V. paradoxus* VAI-C on acyl-HSLs and other compounds[a]

Growth substrate	Yield (g · mol^{-1})	Doubling time (h)
3OC6-HSL	95	3.5
C4-HSL	70	25
C6-HSL	98	32
C8-HSL	114	ND[b]
C10-HSL	170	ND
C12-HSL	197	ND
3-Hydroxybutyrate	53	3.5
Succinate	39	3.5
Isoleucine	68	4.5
Homoserine	57	6
Threonine	35	7
DL-Pantothenate	52	4
Glucose	71	14

[a] The data presented are the average of three or more separate determinations. Other substrates utilized were C14-HSL, 4-hydroxybutyrate, polyoxyethylene-20-sorbitan monooleate (i.e., Tween 80), poly[(R)-3-hydroxybutyrate], glycerol, formate, methionine, propanol, folate, proline, and α-ketobutyrate. Substrates not utilized were L-HSL, γ-butyrolactone, DL-pantolactone, DL-homocysteine thiolactone, *N*-acetyl-L-homocysteine thiolactone, *N*-acetyl-L-aspartate, hexanoate, and $H_2 + CO_2 + O_2$.

[b] ND, not determined (see Materials and Methods).

but not the HSL is used as an energy source. Growth was quite slow on C14-HSL, the longest and least soluble of the acyl-HSLs tested.

For the purposes of comparison, we have determined the molar yields and doubling times exhibited by *V. paradoxus* VAI-C growing on a number of other substrates (Table 1). Of note: growth occurred with homoserine as the sole energy source, but not with L-HSL, γ-butyrolactone, DL-homocysteine thiolactone, or *N*-acetyl-L-homocysteine thiolactone. Growth on acyl-HSLs was comparable to that with many other energy sources in terms of rate and yield on a per carbon basis.

The molar growth yield studies (Fig. 5) indicate that the HSL ring moiety of an acyl-HSL does not serve as an energy source. Nevertheless, the ring may be degraded. To gain insights into the fate of the HSL moiety, we grew cultures with C4-HSL radiolabeled in ring carbon 1. With C4-HSL as the sole source of energy and NH_4Cl as a source of nitrogen, nearly half of the radiolabel was recovered as $^{14}CO_2$. The production of $^{14}CO_2$ demonstrates the cleavage of a significant fraction of the homoserine lactone ring. Of the radioactive label that was not recovered as $^{14}CO_2$, most was accounted for in the culture fluid, even after ethyl acetate extraction. We suspected that this might be HSL. To test this hypothesis, we grew a culture on unlabeled C4-HSL. HSL was the only compound detected by quantitative amino acid analysis (Fig. 6). When taken together with the radiolabling experiments, this indicates that about 25% of the HSL in the C4-HSL was recovered as HSL in the culture fluid. These experiments indicate that HSL is an intermediate in the degradation of C4-HSL. Because HSL accumulates in the culture medium, we suggest that acyl-peptide bond cleavage occurs outside of cells or that there is an export system for intracellular HSL.

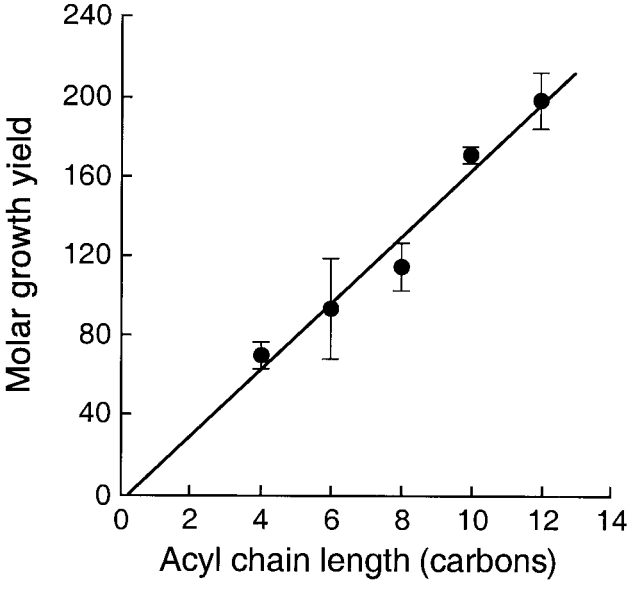

FIG. 5. Molar growth yields of *V. paradoxus* VAI-C on C4-HSL, C6-HSL, C8-HSL, C10-HSL, and C12-HSL. The data points represent the means of four or more separate determinations, and the bars indicate the standard errors.

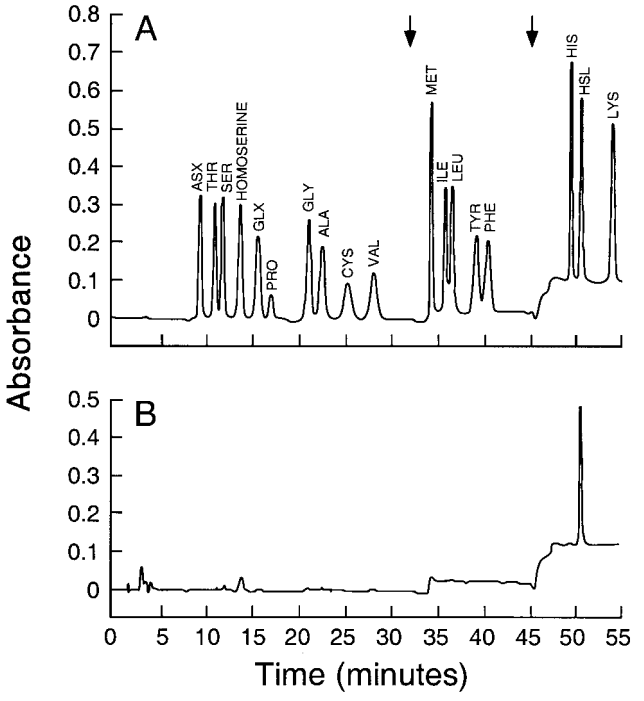

FIG. 6. Amino acid analysis of *V. paradoxus* VAI-C culture fluid. (A) Profile of a mixture of 18 common amino acids, plus homoserine and HSL. (B) Profile of culture fluid analyzed after growth of *V. paradoxus* VAI-C on C4-HSL as the sole source of energy and NH_4Cl as an added nitrogen source. The arrows indicate step changes in the solvent.

Acyl-HSLs as nitrogen sources for growth. *V. paradoxus* VAI-C was capable of growth with acyl-HSLs as the sole source of organic carbon, energy, and combined nitrogen. The acyl group serves as an energy source, and HSL, which is a

TABLE 2. Metabolism of C4-L-[1-^{14}C]HSL by *V. paradoxus* VAI-C

Nitrogen source	Radioactivity (10^3 dpm)[a]				% Recovery
	Consumed	CO_2	Culture fluid	Cells	
NH_4^+ + C4-HSL	625	285 (46)	201 (32)	24 (4)	82
C4-HSL	618	466 (76)	8 (1)	19 (3)	80

[a] Values in parentheses are the amount recovered as a percentage of the amount consumed.

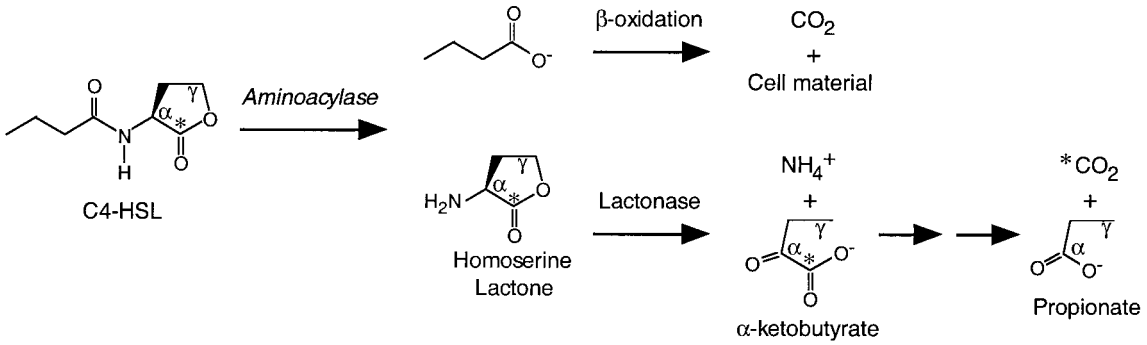

FIG. 7. Hypothetical C4-HSL degradation pathway. The asterisks indicate the position of the ^{14}C-labeled carbon.

product of acyl-HSL metabolism, can serve as a sole source of combined nitrogen for growth. Furthermore, the HSL ring is metabolized even though it does not appear to be utilized as an energy source (Table 2). Thus, we wanted to study growth of the isolate with succinate as an energy source and acyl-HSLs as the only source of nitrogen. Both C4-HSL and 3OC6-HSL served as a nitrogen source. Homoserine and HSL also served as nitrogen sources, but only the acyl-HSLs and homoserine would serve as the sole energy and nitrogen substrate for growth. The doubling times with homoserine, HSL, or an acyl-HSL as the nitrogen source were slow compared to doubling times with NH_4Cl (about 30 h compared to 4 h). However, final cell yields with excess succinate were comparable to the cell yield with NH_4Cl (data not shown). When ^{14}C-labeled C4-HSL was used as a nitrogen source, nearly all of the radioactivity was recovered as CO_2 (Table 2). We do not know whether CO_2 release is an early or late step in the ring cleavage process, but we conclude that ring cleavage and CO_2 release occur prior to utilization of the nitrogen. Whether any HSL carbon, or only its amino group, is incorporated into cellular proteins remains unanswered.

DISCUSSION

We have isolated a bacterium capable of degrading and growing on a number of different acyl-HSLs. This organism appears to be a strain of *V. paradoxus*, and it was able to utilize acyl-HSLs as both energy and nitrogen sources. Apparently only the acyl group is used in energy generation (Table 1 and Fig. 5). In fact, HSL is produced by cultures growing on C4-HSL (Fig. 6). Thus, we believe that the first step in the metabolism of acyl-HSLs involves a carboxypeptidase or aminoacylase that releases the fatty acid from HSL (Fig. 7). By using C4-L-[1-^{14}C]HSL, we showed that *V. paradoxus* cleaves the HSL ring (Table 2). Because the other carbons in the C4-HSL were not labeled, we do not know their metabolic fate.

Although further studies are required to elucidate the pathway of acyl-HSL metabolism, we can speculate that after hydrolysis of the acyl-amide bond, the next step in the degradative pathway might involve the action of a novel α, γ-eliminating deaminase-lyase yielding α-ketobutyrate and NH_4^+ (Fig. 7). Alternatively, a lactone hydrolase (14, 16) could convert HSL to homoserine. However, the latter hypothesis seems inconsistent with the observation that *V. paradoxus* VAI-C utilized homoserine, but not HSL (added exogenously or derived from acyl-HSLs), as an energy source.

The acyl-HSL-degrading activity of *V. paradoxus* VAI-C expands the list of diverse metabolic traits exhibited by members of this bacterial species. Many strains of the species can grow via $H_2 + CO_2 + O_2$ chemolithoautotrophy (3). Other capabilities of strains of *V. paradoxus* include degradation of bioplastics (31), involvement in the dechlorination of xenobiotic compounds (7), and the ability to accumulate the rare metal yttrium (15). Of particular interest, strain VAI-C shares with other *Variovorax* isolates the ability to grow on acyl-amide bond-containing vitamins such as pantothenate (pantoyl-*N*-3-alanine) and folate (pteroyl-*N*-glutamate). The degradation of these vitamins requires two different amino acid-specific carboxypeptidases (10, 11). We believe it is likely that *V. paradoxus* contains an additional acyl-HSL-specific carboxypeptidase.

Acyl-HSLs have received considerable attention as quorum-sensing signals and key regulators of the community behavior of a number of genera of *Proteobacteria* (9, 27, 33). Many acyl-HSLs are quite stable in mildly acidic or neutral pH environments. However, there is no evidence that they accumulate in such environments. If they accumulated over long periods of time, their function as quorum-sensing signals would be disarmed. The signal concentration would not reflect cell number after fluctuations in population density. An intriguing question arises from the identification of bacteria with acyl-HSL-degrading capabilities. Can acyl-HSL-degrading bacteria influence the gene expression of quorum-sensing bacteria when both groups intermingle with or reside in close proximity to each other? This report, along with the report by Dong et al. (4) on a *Bacillus* isolate exhibiting acyl-HSL-inactivating activity, should open up investigations of the metabolic pathway for acyl-HSL degradation, the genetics of acyl-HSL degradation, and the ecological significance of acyl-HSL degradation. As pointed out by Dong et al.(4), the application of acyl-HSL degradation could have value in control of specific plant and animal diseases that are caused by bacteria that employ these quorum-sensing signals to control virulence or biofilm formation. Conversely, there has been interest in exploiting beneficial quorum-regulated activities such as the inhibition of pathogenic fungi around the roots of plants (8, 34). It may be useful to consider biological signal degradation as one factor decreasing the potential effectiveness of acyl-HSL-mediated biocontrol regimes.

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