ORIGINAl RESEARCH

A novel L-isoleucine-4′-dioxygenase and L-isoleucine dihydroxylation cascade in Pantoea ananatis

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Abstract
A unique operon structure has been identified in the genomes of several plant- and insect-associated bacteria. The distinguishing feature of this operon is the presence of tandem hilA and hilB genes encoding dioxygenases belonging to the PF13640 and PF010014 (BsmA) Pfam families, respectively. The genes encoding HilA and HilB from Pantoea ananatis AJ13355 were cloned and expressed in Escherichia coli. The culturing of E. coli cells expressing hilA (E. coli-HilA) or both hilA and hilB (E. coli-HilAB) in the presence of L-isoleucine resulted in the conversion of L-isoleucine into two novel biogenic compounds: L-4′-isoleucine and L-4,4′-dihydroxyisoleucine, respectively. In parallel, two novel enzymatic activities were detected in the crude cell lysates of the E. coli-HilA and E. coli-HilAB strains: L-isoleucine, 2-oxoglutarate: oxygen oxidoreductase (4′-hydroxylation) (HilA) and L-4′-hydroxyisoleucine, 2-oxoglutarate: oxygen oxidoreductase (4-hydroxylation) (HilB), respectively. Two hypotheses regarding the physiological significance of C-4(4′)-hydroxylation of L-isoleucine in bacteria are also discussed. According to first hypothesis, the L-isoleucine dihydroxylation cascade is involved in synthesis of dipeptide antibiotic in P. ananatis. Another unifying hypothesis is that the C-4(4′)-hydroxylation of L-isoleucine in bacteria could result in the synthesis of signal molecules belonging to two classes: (5H)-furanones and analogs of N-acyl homoserine lactone.

Introduction
The C-4-hydroxylation of free L-amino acids is a recently discovered secondary metabolism phenomenon in bacteria. L-Isoleucine-4-dioxygenase (IDO; systematic name: L-isoleucine, 2-oxoglutarate: oxygen oxidoreductase [4′-hydroxylation]), a member of the Pfam PF10014 family, was first found in Bacillus thuringiensis (Kodera et al. 2009). Since its discovery, this enzyme has been thoroughly characterized (Hibi et al. 2011) and is now used for the biotechnological synthesis of 4-hydroxyisoleucine, a natural nonproteinogenic amino acid with insulinotropic biological activities (Smirnov et al. 2010). Subsequent investigations revealed the existence of a novel subfamily of bacterial dioxygenases belonging to the PF10014 (BsmA) family (Weber et al. 2010) that accept free L-amino acids as substrates in vivo. L-leucine, L-isoleucine, and L-threonine were found to be hydroxylated by the PF10014 members, producing 4-hydroxyleucine, 4-hydroxyisoleucine, and 4-hydroxythreonine, respectively (Smirnov et al. 2012). A detailed analysis of the genetic organization of PF10014 members revealed a unique operon structure (designated the Hil operon, from the hydroxylation of isoleucine) in the genomes of several gram-negative plant pathogens belonging to the Pantoea and Pseudomonas genera (Smirnov et al. 2012) (Fig. 1).
Figure 1. The structure of the Hil operons of plant- and insect-associated bacteria. The putative operon structures were deduced from the analysis of the following genomes. (A) Bacillus thuringiensis sp. 2e2 (Smirnov et al. 2012); (B) Pantoea ananatis AJ13355 (Hara et al. 2011), P. ananatis LMG 20103 (De Maayer et al. 2010), P. ananatis LMG 5342 (De Maayer et al. 2012), and Rahnella aquatilis CIP 78.65 (Martinez et al. 2012); (C) Pseudomonas syringae pv. phaseolicola 1448A (Joardar et al. 2005); (D) Pseudomonas savastanoi pv. glycinea (Qi et al. 2011); and (E) Xenorhabdus nematophila ATCC 19061. The distinguishing feature of this operon is the presence of a “core” of five genes: HilR – a putative LysR-type transcriptional regulator (Pfam family PF00126); HilB – a putative extracellular solute-binding protein (PF00497); HilA – a putative dioxygenase (PF13640); HilP – a dioxygenase (PF10014) that is a close homologue of L-isoleucine-4-dioxygenase (IDO) (Smirnov et al. 2012); and HilC – a short-chain dehydrogenase (PF00106) that is a close homologue of 2-amino-3-methyl-4-ketopentanoic acid (AMKP) reductase (AR). Other genes included in the Hil operon are bacterium specific: RhtA – putative exporters belonging to the Rht family (Livshits et al. 2003); HilD – putative ATP-dependent carboxylate-amine ligase (PF13535); HilE – putative major facilitator superfamily (MFS) transporter (PF07690); and PALP – putative pyridoxal-phosphate dependent enzyme (PF00291). The arrows corresponding to the genes are not drawn to scale to enable all operons to be fit into one figure. The arrows indicating homologous proteins have the same shading.

The distinguishing feature of this operon is the presence of tandem hilA and hilB genes encoding putative dioxygenases belonging to the PF13640 and PF10014 Pfam families, respectively. Previously, we found that HilB from Pantoea ananatis AJ13355 also catalyzes the C-4-hydroxylation of l-isoleucine, but the $K_M$ value deduced from the kinetic analysis of this reaction was much greater than that determined for IDO, suggesting that l-isoleucine is not a natural substrate of HilB (Smirnov et al. 2012). In contrast to IDO, HilB is coexpressed with a putative dioxygenase, HilA. Moreover, in some bacteria, there is translational coupling between HilA and HilB, suggesting that these enzymes form a complex that catalyzes two consecutive reactions and implying that the natural substrate of HilB is a product of the reaction catalyzed by HilA. In this study, we provided evidence supporting this hypothesis and characterized the enzymatic activities of HilA/HilB from P. ananatis AJ13355.

Materials and Methods

Bacterial strains, plasmids, and oligonucleotides

The bacterial strains, plasmids, and oligonucleotides used in this study are listed in Tables 1 and 2.

Cloning of the hilA and hilB genes from P. ananatis AJ13355

To construct the pETAC-HilA and pETAC-HilAB plasmids, the following DNA fragments were amplified using the chromosomal DNA of the P. ananatis AJ13355 strain as a template: (1) a 935-bp fragment, “HilA,” using the primers svs191 and svs191*; and (2) a 1732-bp fragment, “HilAB,” using the primers svs191 and svs190. The resulting fragments were digested with Xbal and SacI and then ligated into a pETAC-ilvA/Xbal-SacI vector to yield the target plasmids.

Low-scale biotransformation of l-isoleucine into l-4,4′-dihydroxyisoleucine (4′-HIL) and l-4,4′-dihydroxyisoleucine (4,4′-DIHIL)

All constructed recombinant plasmids harboring the hilA/ hilB genes and pUC19 as a “control” plasmid were introduced into the Escherichia coli TG1 strain. The resulting plasmid strains were cultivated in 2 mL of T1 medium (50 mmol/L KH2PO4 [pH 7, adjusted with KOH], 20 mmol/L NH4Cl, 2 mmol/L MgSO4, 1 mmol/L IPTG (isopropyl β-D-thiogalactopyranoside), 3 g/L l-isoleucine, 200 mg/L ampicillin, 10% [v/v] LB-broth, 10 g/L glucose, 1.25 g/100 mL chalk) at 32°C for 48 h in 20 mL test tubes with vigorous shaking. The final culture broth was analyzed using TLC (thin layer chromatography) and HPLC (high-performance liquid chromatography) analyses.

Large-scale preparation and purification of 4′-HIL

The TG-HilA strain was cultivated in 750 mL flask containing 50 mL of LB broth at 37°C until $A_{540} = 1$ was reached. HilA expression was induced by adding IPTG to a final concentration of 1 mmol/L. Then, the induced cells were cultivated at 34°C for approximately 2.5 h. The obtained “active” biomass was harvested by centrifugation and resuspended in 5 mL of reaction buffer (50 mmol/L KH2PO4 pH 7, adjusted with NaOH; 20 mmol/L NH4Cl; 2 mmol/L MgSO4; 1.25 g/100 mL chlk; 1 mmol/L IPTG; 100 mmol/L l-isoleucine; and 100 mmol/L α-ketoglutarate). The resulting suspension was cultivated at 32°C for
48 h with vigorous shaking. Then, the cells were precipitated by centrifugation, and the resulting culture broth was filtered using a 0.45 μm CHROMAFIL Xtra CA-45/25 syringe filter and applied to a DOWEX 50WX2 column (1.3 × 16 cm) equilibrated with 30 mmol/L ammonia solution. An isocratic elution was performed using the same ammonia solution at a 1.5 mL/min elution rate, and 3 mL elution fractions were collected. Fractions containing 4′-HIL were pooled, lyophilized, and stored at −20°C until use.

Large-scale preparation and purification of L-4,4′-DIHIL

The TG-HilAB strain was cultivated in LB medium (2 × 100 mL in 0.75 L flasks) at 37°C until OD$_{555}$ = 1 was reached. The induction of HilA$^{AA}$ and HilB$^{AA}$ synthesis was carried out by IPTG adding to final concentration of 1 mmol/L. Then, the culture was incubated at 32°C for approximately 16 h with vigorous shaking. Then, the following procedure was repeated twice: a 2.5 mL aliquot of culture broth was passed through a 0.45 μm CHROMAFIL Xtra CA-45/25 syringe filter and then applied to a DOWEX 50WX2 column (0.8 × 16 cm) equilibrated with 30 mmol/L ammonia solution. An isocratic elution was performed using the same ammonia solution at a 1.5 mL/min elution rate, and 3 mL elution fractions were collected. Fractions containing L-4,4′-dihydroxyisoleucine were pooled, lyophilized, and stored at −20°C until use.

Analytical methods

L-Isolucine hydroxylation was monitored by TLC analysis (n-BuOH:acetic acid: water = 12:3:5) using ninhydrin.

A high-pressure chromatography system with an 1100 series spectrofluorimeter (Agilent, Santa Clara, CA) was
used for the HPLC analysis of the hydroxylation products. The detection used an excitation wavelength of 250 nm and an emission wavelength range of 320–560 nm. The amino acid derivative formation and separation were performed according to the manufacturer’s recommendations (Waters). Separation by the AccQ-tag method was performed using an XBridge C18 column (2.1 × 150 mm, 5 μm) at 30°C with a flow rate of 0.3 mL/min. The mobile phases were AccQ-tag eluent A (eluent A) and acetonitrile (eluent B), and the flow rate of the eluent was 0.3 mL/min. The eluent gradients were 10–25% (v/v) B over 0–20 min, 60% (v/v) B for 20.1–23 min, and 10% (v/v) for 23.1–30 min.

LC/ESI-MS and 1H(13C)-NMR analyses of purified 4′-DHIL and 4,4′-DIHIL were performed as was described in (Hibi et al. 2011).

**Purification of HilA**

Cells of the TG-HilA strain from an LB-agar plate were inoculated into 1 L of LB broth (5 × 200 mL in 750 mL flasks) supplemented with Ap (100 mg/L), and the cultures were grown at 34°C until OD540 = 1 was reached. Then, protein expression was induced by adding IPTG (1 mmol/L) and incubating the culture for an additional 2 h. Then, the cells were harvested and stored at −70°C until use.

**Step 1.** The cells were thawed and resuspended in 40 mL of buffer A (50 mmol/L Tris pH 7, 50 mmol/L NaCl, 1 mmol/L DTT, 1 mmol/L EDTA). The cells were disrupted by two passages through a French pressure cell (maximum pressure, 2000 Psi) followed by centrifugation (14000 g, 4°C, 20 min) to remove the cell debris.

**Step 2.** Forty milliliters of the protein preparation obtained from Step 1 was applied to a DEAE-Sepharose-Fast Flow (Sigma) column (1.6 × 25 cm) equilibrated with buffer A. The unbound (flow-through) fraction was collected.

**Step 3.** The protein obtained in Step 2 was precipitated by adding (NH4)2SO4 to 40% saturation and then resuspended in 2 mL of buffer A.

**Step 4.** One milliliter of the protein preparation obtained from Step 3 was applied to a Superdex 200 HR 10/30A column equilibrated with buffer A. An isocratic elution was performed at a flow rate of 0.5 mL/min. Each 1-mL fraction was collected. Active fractions were pooled and stored at −70°C until use.

**Purification of HilB**

We performed expression and purification of his6-tagged HilB protein as was described previously in (Smirnov et al. 2012).

**Determination of dioxygenase activity**

The reaction mixture (50 μL) contained 100 mmol/L HEPEP (pH 7 adjusted with KOH), 5 mmol/L Ile, 10 mmol/L α-ketoglutarate, 5 mmol/L l-ascorbate, 5 mmol/L FeSO4, and an aliquot of the protein preparation (20–50 μg of total protein). The purified l-4′-hydroxyisoleucine (≈5 mmol/L, see above) was used in reaction with purified HilB instead l-isoleucine. The reaction was carried out at 34°C for 1 h with vigorous shaking. The reaction products (l-4′-hydroxyisoleucine and l-4,4-dihydroxyisoleucine) were detected using TLC and/or HPLC analysis.

**Results**

Because HilA and HilB are dioxygenases, it could be hypothesized that these enzymes catalyze the consecutive hydroxylation of an unknown substrate. Previously, we found that the substrate specificity profile of HilB for free amino acids is similar to that of IDO (for which l-isoleucine is a natural substrate) (Smirnov et al. 2012). Both enzymes catalyze the oxidation of l-methionine and the C-4-hydroxylation of l-leucine and l-isoleucine but exhibit different kinetic parameters for these reactions. The KM and Vmax values of both enzymes were similar for l-leucine but were very different for l-isoleucine and l-methionine. These data suggested that HilB could catalyze the C-4-hydroxylation of an unknown derivative of l-isoleucine. We hypothesized that the unknown compound is l-isoleucine hydroxylated by HilA at another C-position.

To test this hypothesis, we first investigated the in vivo activity of HilA with l-isoleucine. For this purpose, we cloned the hilA gene from P. ananatis AJ13355 into a multicopy vector under the transcriptional control of the strong P_tac promoter. The constructed plasmid was introduced into E. coli TG1 cells and the expression of HilA protein was confirmed by SDS-PAGE analysis of crude cell lysate of the resulting TG-HilA strain (Fig. 2A). Then, the TG-HilA strain was cultivated in synthetic medium supplemented with l-isoleucine. The TG1 strain harboring the pUC19 vector was also cultivated as a negative control under the same conditions. TLC and HPLC analyses of the resulting culture broth were then performed. These analyses showed that l-isoleucine was completely absent from the culture broth of the TG-HilA strain, and an unknown amino group-containing compound (C3) was detected (Fig. 3A and D). In the case of the TG-C strain, the l-isoleucine concentration was unchanged, and no new compounds were synthesized (Fig. 3B). These data explicitly suggest that l-isoleucine was converted into C3 due to the enzymatic activity of HilA. To further test this hypothesis, we investigated the l-isoleucine-dioxygenase activity of a crude cell lysate of TG-HilA. For this purpose,
we performed a complete dioxygenase reaction and a set of control reactions, each lacking one essential component (L-isoleucine, α-ketoglutarate, L-ascorbate, or FeSO₄). We found that the synthesis of C3 was strongly dependent on the presence of L-isoleucine, α-ketoglutarate, and FeSO₄ (Table 3). We purified HilA from crude cell lysate of TG-HilA and investigated its dioxygenase activity with L-isoleucine in the same way. Resulting data were identical to those obtained with crude cell lysate of TG-HilA (Fig. 2B, Table 3). In addition, we confirmed dioxygenase activity of HilA by using a generic assay that uses succiny L-coenzyme A synthetase, pyruvate kinase, and lactate dehydrogenase to couple the formation of the product succinate to the conversion of reduced nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide (Luo et al. 2006). To determine the chemical structure of C3, it was purified and subjected to ¹H-NMR, ¹³C-NMR, and ESI-MS analyses (Table 4). These data allowed us to identify C3 as 4′-hydroxyisoleucine and HilA as an L-isoleucine, 2-oxoglutarate: oxygen oxidoreductase (4′-hydroxylating) (Fig. 4B). In addition to L-isoleucine, L-valine, and L-methionine were transformed by purified HilA (although the enzyme exhibited low activity with these substrates), resulting in the formation of 4-hydroxyvaline (identified...
Table 3. Synthesis of C2 and C3 by the dioxygenase reaction with different substrate compositions.

| Enzyme     | Reaction | A  | B  | C  | D  | E  |
|------------|----------|----|----|----|----|----|
| HILα1      |          | C3 | nd | nd | C3 | nd |
| HILαB1     |          | C2 | C3 | nd | nd | C2, C3 nd |
| HILα2      |          | C3 | nd | nd | C3 | nd |
| HILβ2      |          | C2 | nd | nd | C2, C3 nd |
| HILα/HILβ2 |          | C2 | C3 | nd | nd | C2, C3 nd |

1The crude cell lysates of TG-HILαA and TG-HILαB were used for the in vitro dioxygenase assay.
2The purified HILαA and/or purified his6-tagged HILβ protein were used for dioxygenase assay.
3The reaction mixture contained the following: A, all components; B, C, D, and E, the same as A but without l-isoleucine (l-4,4-dihydroxyisoleucine in reactions with HILβ), α-ketoglutarate, l-ascorbate, and FeSO4, respectively.
4If equimolar concentrations of l-isoleucine and α-ketoglutarate were used in the HILα activity assay, then the formation of both C3 (l-4HIL) and C2 (4,4′-DIHIL) was detected, whereas a twofold excess of α-ketoglutarate resulted in the complete conversion of l-isoleucine into C2 in vivo.
5Not detected.

using 1H NMR and ESI-MS analyses, data not shown) and presumably l-methionine sulfoxide, respectively (Fig. 4C).

Previously, using the method described in (Smirnov et al. 2010), we found that E. coli cells expressing HILβ convert l-isoleucine into 4-hydroxyisoleucine but that this conversion is much less effective than that using IDO (S. V. Smirnov, unpubl. data). Thus, when expressed without HILA, HILβ acts as a minimally effective IDO (Fig. 4A). To determine how HILβ functions in the presence of HILA in vivo, we cloned and expressed a tandem hilA-hilB construct in E. coli (Fig. 2B). The TG-HILαB strain was cultivated in the presence of l-isoleucine, as described above for the TG-HILαA strain. The TLC and HPLC analyses of the resulting culture broth showed that l-isoleucine was completely absent from the culture broth of the TG-HILβ strain, and an unknown amino group-containing compound (C2) was detected (Fig. 3A and C). We identified C2 as 4,4′-DIHIL (Table 4, Fig. 4B). The α-ketoglutarate/Fe2+-dependent synthesis of 1-4,4′-DIHIL from l-isoleucine was detected in the crude cell lysate of the TG-HILβ strain (Table 3). If equimolar concentrations of l-isoleucine and α-ketoglutarate were used in the activity assay, then the formation of both 4′-HIL and 4,4′-DIHIL was detected, whereas a twofold excess of α-ketoglutarate resulted in the complete conversion of l-isoleucine into 1-4,4′-DIHIL. The in vitro investigation of combined dioxygenase activity of purified HILα and HILβ revealed the same results. Finally, the purified HILβ exhibited an α-ketoglutarate/Fe2+-dependent 1-4′-hydroxyisoleucine-4-dioxygenase activity (Table 3).

These data allowed us to identify HILβ as an 1-4′-hydroxyisoleucine, 2-oxoglutarate: oxygen oxidoreductase (4-hydroxylating) (Fig. 4B). Thus, we can conclude that HILα and HILβ catalyze the consecutive hydroxylation of l-isoleucine at the C-4′ and C-4 positions, respectively, to synthesize 1-4′,4-dihydroxyisoleucine (Fig. 4B).

**Discussion**

Many natural pharmaceutically active compounds with anti-cancer, antibiotic, and immunosuppressant activities are produced by microbial secondary metabolism (Demain 1999; Sithranga Boopathy and Kathiresan 2010;
Vaishnav and Demain (2011). In this context, the newly discovered secondary metabolites 4′-HIL and 4,4′-DIHIL may also be used in pharmaceutical biotechnology. Indeed, both compounds are close “chemical homologues” of 4-HIL—a natural bioactive molecule that significantly decreases plasma triglyceride levels, total cholesterol, and fatty acid levels and increases glucose-induced insulin release in human and rat pancreatic islet cells in vitro (Jette et al. 2009). Thus, it would be very interesting to investigate the analogous biological activity of novel forms of hydroxylated L-isoleucine. If these molecules are used by the modern pharmaceutical industry in the future, their synthesis from L-isoleucine can be easily carried out using the method described in (Smirnov et al. 2010).

Plant-associated bacteria (epiphytes and endophytes) serve as an important source of bioactive molecules. These bacteria produce numerous secondary metabolites that are involved in plant–bacterium and interbacterial interactions, including plant pathogenesis and quorum sensing/quenching-related processes (Strobel 2003; Gunatilaka 2006; Pimentel et al. 2011). It is notable that the Hil operon is found exclusively in plant and insect-associated bacteria. Our first insight into the physiological role for this operon is based on the noticeable structural-functional similarity between the Hil operon and the IDO and Bac operons from Bacillus subtilis (Fig. 5).

Abstracting from specific biochemical details, it can be argued that all three operons provide synthesis and excretion of biologically active substances, the effect of which is directed against environmental microbiota. As the toxic compounds are synthesized within the bacterial cell in each case, the special mechanism of self-detoxification is needed to prevent self-killing of bacteria. Thus, from the functional point of view, each operon contains two primary gene groups. First group consists of genes encoding proteins synthesizing toxic substance. Second group includes genes encoding proteins providing self-detoxification of cell.

2-amino-3-methyl-4-ketopentanoic acid (AMKP) synthesized by the B. thuringiensis is a “conditional” antibiotic that inhibits the growth of Escherichia coli, Staphylococcus aureus, Sarcina lutea, B. subtilis, and Pseudomonas aeruginosa growing only in the glucose minimal salts medium. No inhibitory activity was noted when the organisms were grown in a peptone-yeast extract medium, presumably because methionine, L-valine, L-leucine, and L-isoleucine present reversed the inhibition of the growth of the test organisms (Perlman et al. 1977). Thus, the active export of AMKP by RhtA seems to be enough for self-detoxification of B. thuringiensis.

On the contrary, anticapsin is a strong competitive inhibitor of house-keeping enzyme glucosamine synthase (GS). The irreversible inhibition of GS results in the lysis of bacterial or fungal cells. In this case, B. subtilis uses additional mechanism of “autodetoxification”—synthesis of dipeptide bacilysin in which the toxic anticapsin is “hidden” by binding with L-alanine. The antimicrobial activity of anticapsin is then initiated by reuptake and

Figure 4. C-4(4′)-hydroxylation of L-isoleucine in bacteria. (A) The hydroxylation of L-isoleucine (1) by IDO from Bacillus thuringiensis results in the formation of 4-HIL (2). (B) The consecutive dihydroxylation of L-isoleucine catalyzed by HiiAB results in the synthesis of the novel compounds 4′-hydroxyisoleucine (3) and 4,4′-dihydroxyisoleucine (4). (C) The C-4 hydroxylation of L-valine (5) forms L-4-hydroxyvaline (6).
proteolysis of bacilysin by a peptidase to release anticapsin in an environmental target cell.

In the Hil operon, the expression of HilA and HilB is also coupled with the expression of an AR homologue (HilC), suggesting that oxidized 4,4′-DIHIL may be a novel antimicrobial agent. May be the “power” of this compound is comparable with that of anticapsin and may be that is why the Hil operon contains the BacD homologue, putative L-amino acid ligase HilD, thus providing the self-detoxification by mechanism used in B. subtilis (Fig. 5).

Because some “bearers” of the Hil operon (Pantoea, Rahnella, Pseudomonas) are plant epiphytes, further investigation of the Hil operon may be very useful for the
construction of effective biological control agents with a broad activity spectrum against phytopathogenic bacteria (Chen et al. 2009).

It is interesting to note that the C-4(4′)-hydroxylation of L-isoleucine (and other free L-amino acids) could, in theory, result in the synthesis of two classes of signal molecules: 2(5H)-furanones and analogs of N-acyl homoserine lactone (AHL). Lactone formation followed by the Strecker reaction can directly yield 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) (Blank et al. 1996) (Fig. 6A). An analogous pathway may be proposed for the synthesis of the corresponding 2(5H)-furanones and N-acyl homoserine lactone derivatives from 4′-HIL and 4,4′-dihydroxyisoleucine (Fig. 6B).

Figure 6. The C-4(4′)-hydroxylation of free L-isoleucine as a putative pathway for the synthesis of novel signaling molecules. (A) The lactonization (a) of 4-HIL followed by oxidative deamination (b) or amide bond formation (c) could result in the synthesis of 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) or N-acyl homoserine lactone analogs. (B) An analogous pathway may be proposed for the synthesis of the corresponding 2(5H)-furanones and N-acyl homoserine lactone derivatives from 4′-HIL and 4,4′-dihydroxyisoleucine.

bacteria synthesize volatile, strong-smelling insect attractants to facilitate the spread of these bacteria.

The lactonization of C-4(4′)-hydroxylated free L-amino acids also enables the synthesis of AHL derivatives. This putative pathway could include lactonization and the formation of an amide bond between the carboxyl group of the “tail” (RCOOH) compound and the amino group of the “head” lactone molecule (Fig. 6). The resulting compounds could be used for the modulation of AHL and AI-2 quorum sensing pathways (Galloway et al. 2011). In this context, it is interesting to note that the ATP-dependent carboxylate-amino ligase HilD could be involved in the ligation of 4′-HIL and/or 4,4′-DIHIL with an unknown “tail” carboxylate.

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Conflict of Interest

None declared.

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