Curr Microbiol
DOI 10.1007/s00284-013-0490-7

Sequencing, Characterization, and Gene Expression Analysis of the Histidine Decarboxylase Gene Cluster of *Morganella morganii*

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Received: 25 March 2013 / Accepted: 2 October 2013

Abstract The histidine decarboxylase gene cluster of *Morganella morganii* DSM30146^T was sequenced, and four open reading frames, named *hdcT1*, *hdc, hdcT2*, and *hisRS* were identified. Two putative histidine/histamine antiporters (*hdcT*1 and *hdcT2*) were located upstream and downstream the *hdc* gene, codifying a pyridoxal-P dependent histidine decarboxylase, and followed by *hisRS* gene encoding a histidyl-tRNA synthetase. This organization was comparable with the gene cluster of other known Gram negative bacteria, particularly with that of *Klebsiella oxytoca*. Recombinant *Escherichia coli* strains harboring plasmids carrying the *M. morganii hdc* gene were shown to overproduce histidine decarboxylase, after IPTG induction at 37 °C for 4 h. Quantitative RT-PCR experiments revealed the *hdc* and *his*RS genes were highly induced under acidic and histidine-rich conditions. This work represents the first description and identification of the *hdc*-related genes in *M. morganii*. Results support the hypothesis that the histidine decarboxylation reaction in this prolific histamine producing species may play a role in acid survival. The knowledge of the role and the regulation of genes involved in histidine decarboxylation should improve the design of rational strategies to avoid toxic histamine production in foods.

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Introduction

Histamine and other biogenic amines are low molecular weight organic bases formed in foods by microbial decarboxylation of the corresponding amino acids, through the action of inducible amino acid decarboxylases that replace the α-carboxyl groups of specific amino acid substrates with a proton consumed from the cytoplasm. Consumption of internal protons and release of reaction products, (di- or tri-amines), provides local buffering of the extracellular environment [8, 25].

Histamine is one of the main concerns in fisheries products. It originates from the action of a histidine decarboxylase (Hdc) in fresh fish that have high amount of histidine, such as bonito and tuna, causing the notorious foodborne intoxication (histamine fish poisoning or HFP), also known as Scombroid poisoning [17, 21, 29]. It is, therefore, important to prevent this accumulation through the use of different preservation techniques [14, 19, 20, 22] to reduce the health risk related to fish product consumption. Today, with the development of new molecular tools, comprising DNA sequencing-based methods and reverse transcription quantitative PCR (RT-qPCR) techniques [1, 6, 13], it is possible to further contribute to the study of histamine-forming bacteria.

Bacterial strains implicated in HFP include enteric bacteria, such as *Morganella morganii* [32], *Raoultella planticola*, *Enterobacter aerogenes* [16], and marine bacteria, such as *Photobacterium phosphoreum* [23] and *Photobacterium damselae* subsp. *damselae* [18]. However, despite the clinical significance of these strains, only the marine *Photobacterium* spp. has been studied in detail at molecular level. For *P. phosphoreum*, a sequence analysis of the DNA corresponding to the *hdc* gene revealed an open reading frame coding for a pyridoxal-5′-phosphate-dependent Hdc,

Published online: 17 November 2013

whose amino acid sequence showed high similarity with species of the family *Enterobacteriaceae*. In addition, a putative amino acid permease gene (*ydj*E) and a histidine-tRNA synthetase gene (*his*S) were found in the *hdc* flanking regions [23]; for *P. damselae* a histidine/histamine antiporter (*hdc*T), the histidine decarboxylase (*hdc*A) and a histidyl-tRNA synthetase (*his*RS) were detected. Northern blot analysis and RT-PCR revealed high levels of mono- and bicistronic transcripts of *hdc*A, *hdc*T, and *his*RS under conditions of low pH and histidine excess, suggesting these genes are part of an operon [18]. For *R. planticola* and *E. aerogenes,* data available in the literature refer to the sequence of the *hdc* genes encoding for an inducible pyridoxal–phosphate-dependent histidine decarboxylase, but any studies were carried out on *hdc*-related genes [16].

The aim of the present study was to investigate the organization of the histidine decarboxylase gene cluster of *M. morganii*. This microorganism is considered one of the most prolific histamine former in fish [4, 9]. However, little information is available in the literature; it principally refers to the characteristics of the Hdc, a pyridoxal-5′-phosphate (PLP) dependent enzyme whose deduced amino acid sequence shows the catalytic residue lysine at position 232 with which pyridoxal 5-phosphate forms an internal aldimine (Schiff base linkage), essential for enzyme activity [17, 31, 32]. Until now, no information concerning the organization and localization of histamine-related genes in *M. morganii* is available.

By employing a plasmid-DNA library, the *hdc* gene cluster of *M. morganii* DSM30164^T was isolated, sequenced, and expressed in *Escherichia coli*. Further, RT-qPCR experiments were carried out to evaluate the different gene expression in response to environmental conditions.

Materials and Methods

Bacterial Strains, Growth Conditions and Plasmids

Morganella morganii DSM30164^T was routinely grown in Luria–Bertani (LB) medium at 37 °C for 16 h. For RT-qPCR experiments, a minimal medium (Tx) containing NaCl 10 g/l and yeast extract 1 g/l was used. *E. coli* DH5αF′[F′/endA1 hsdR17(rk-mk+) supE44 thi-1 recA1 gyrA(-Na1r) relA1 Δ(lacIZYA-argF)U169 deoR (Φ80dlac Δ(lacZ)M15); Promega, Spain] was used for all DNA manipulations. *E. coli* BL21(DE3) pLysS (Promega) competent cells were used for expression in pURI3 vector. This plasmid encodes expression of a leader sequence consisting of a N-terminal methionine followed by three spacer amino acids, a six histidine affinity tag, a spacer glycine residue, and the five amino acid enterokinase (EK) recognition site, under control of the T7 RNA polymerase φ promoter, which can be induced at high levels of isopropyl-β-D-thiogalactopyranoside (IPTG) [5]. *E. coli* strains were grown in LB medium at 37 °C by shaking at 200 rev/min. When required, ampicillin was added to the medium at 100 μg/ml. For the sequencing of the flanking regions of the *hdc* gene, a DNA library was build, using the vector pUC19 (Takara Bio Inc., Japan). All these strains were conserved at −80 °C in 15 % glycerol stock. Plasmids were conserved at −20 °C.

DNA Manipulation and Library

Chromosomal DNA, extracted as previously described [10], and vector pUC19 were digested with different restriction enzymes (*Hind*III, *Pst*I, *Xba*I, *Sac*I, *Eco*RI—Roche, Spain, *Bbu*I—Promega, *Sal*I, *Sma*I—Takara, *Bam*HI—BioLabs, Spain). All DNA manipulations were performed according to standard procedures [27]. PCR was performed using 1.0 U of AmpliTaq Gold DNA polymerase (Roche) in 50 μl reaction mixture, containing 5 μl of GeneAmp 10× PCR Gold Buffer (Roche), 5 μl of $MgCl_2$ (25 mM), 5 μl of dNTP (2.5 mM each), and 0.3 μM of primers. Amplification was carried out using a PCR-Mastercycler 96 (Eppendorf, Germany) programmed as follows: an initial denaturation step at 95 °C for 10 s, followed by 30 cycles of 95 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min, and a final elongation step at 72 °C for 10 min. All the amplifications were done using a primer annealing into the plasmid and a primer located at the end of the known region (Table 1). Amplified DNA fragments, separated by 0.7 % agarose gel, were extracted, purified using the QIAquick Gel Extraction Kit (Qiagen, Spain), and sequenced in an ABI Prism 3700TM DNA sequencer (Applied Biosystems, USA).

Heterologous Expression of *hdc* in *Escherichia coli*

To clone into the pURI3 expression vector, the *hdc* gene from *M. morganii* was amplified using primers 1046 and 1047 (Table 1). PCR was performed in a 50 μl reaction mixture, containing 100 ng of chromosomal DNA, 10 μl of 5× PrimeSTAR Buffer (Mg^{2+} plus, Mg^{2+} concentration is 5 mM), 4 μl of dNTP Mixture (2.5 mM each) (Takara), 0.3 μM of each primer, and 1.25 U PrimeSTAR HS DNA polymerase (Takara). Amplification was carried out using a thermal protocol composed by 30 cycles of 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 90 s, followed by a final extension at 72 °C for 10 min. After amplification, the 1.2 kb PCR product was gel purified and inserted into pURI3 vector by using the enzyme restriction- and ligation-free cloning strategy described previously [5]. Briefly, the purified PCR products were used as homologous primer pair

Table 1 Oligonucleotides designed and used in this study and their applications

Primer	Sequence (5′–3′)	Purpose
1046	CATCATGGTGACGATGACGATAAGATGACTCTGTCTATCAATGATCAAA	Cloning in pURI3 and amplification of *hdc*
1047	AAGCTTAGTTAGCTATTATGCGTATTATGCCGCGTGTAAGTTAAAATCC	Cloning in pURI3 and amplification of *hdc*
1044	GTCATAAAGTTGTAGCCAATA	DNA library
1054	CGTCAGAACGCGTCTGGAGAGAAC	DNA library
1233	ACGGGATAACAATTTCACACAGGA	DNA library
1224	CGCCAGGGTTTTCCCAGTCACGA	DNA library
1053	GCAGTGATAATGGCAGTGGTATC	DNA library
1064	CGCCACTCTGAGCCTGATTG	DNA library
1069	CTGGAACATATTTTAATGCAG	DNA library
1070	GGTACGCTGATATTTATTATTG	DNA library
1093	TATGATGCCAGTGCGTCGCATC	DNA library
Mm453F [10]	TTTCAGTCGGGAGGAAGGTG	RT-PCR
Mm631R [10]	GGGGATTTCACATCTGACTC	RT-PCR
hdcRT fw	ACTCAATCGGTGTTTCCGGC	RT-PCR
hdcRT rv	TGTGACCGTTACGTGAACCG	RT-PCR
hisRS RT fw	CGGTGAATCCACCGATATCG	RT-PCR
hisRS RT rv	TGAGTGAACTGACGCAGACG	RT-PCR

in a PCR reaction using the pURI3 expression vector as template [7]. The reaction mixture (50 µl) contained 300 ng of the purified fragment, 50 ng of the recipient plasmid, 10 µl of 5× PrimeSTAR Buffer (Takara), 4 µl of dNTP Mixture (2.5 mM each) (Takara), and 1.25 U PrimeSTAR HS DNA polymerase (Takara). After 30 amplification cycles (95 °C for 30 s, 55 °C for 5 s and 72 °C for 9 min), the PCR product was digested with *Dpn*I, that exclusively restrict methylated DNA, and later, with *Not*I that only cuts the original copies of pURI3 vector. *E. coli* DH5α cells were transformed directly with the digestion product.

The plasmid was then transferred, for protein production, to the host *E. coli* BL21(DE3), grown at 37 °C in LB medium containing 100 µg/ml ampicillin. When the culture reached 0.5 OD_{600nm}, IPTG (0.4 mM final concentration) was added, and the culture was further incubated at different temperatures (16, 22, or 37 °C) for different times (4 or 16 h). Cells were harvested by centrifugation (10,000×*g*, 10 min, 4 °C), washed with 50 mM phosphate buffer (PB, pH 6.5), and mechanically disrupted by three French Press passages (1,000 psi). The insoluble fraction was separated by centrifugation (10,000×*g*, 30 min, 4 °C), and the supernatant was quantified using the Bio-Rad protein assay (Biorad, Madrid). The protein extracts were resuspended in SDS-PAGE (Laemmli) sample buffer, boiled for 5 min, then separated on a 10 % polyacrylamide gel in Tris–glycine-SDS buffer. Gels were stained with Coomassie brilliant blue G-250 (Sigma-Germany).

For protein purification *E. coli* BL21 pURI3hdc cells were harvested by centrifugation, resuspended in PBS (50 mM phosphate buffer, NaCl 300 mM, pH 6.5), and disrupted. The soluble fraction was applied to a Talon Superflow metal affinity column (Clontech, Spain), and the enzyme was eluted by applying a continuous gradient of imidazole concentration, from 10 to 150 mM. Fractions containing the eluted Hdc were pooled and dialysed against PBS. Hdc activity was assayed in 130 µl of PBS, containing 0,043 M of acetic acid, 3.6 mM L-histidine (Sigma), 400 µM PLP (Sigma-Aldrich), and 50 µg of the purified protein. After incubation at 37 °C for 10 min and 1 h, histamine formed was derivatized and detected by TLC as described by García-Moruno et al. [12].

RNA Extraction and Preparation

RNA extraction was performed using the NucleoSpin RNA II extraction kit (Macherey–Nagel GmbH, Germany), in *M. morganii* cultures grown either in Tx minimal medium or LB rich medium, both prepared at different pH values (7.0 or 4.5) and in the presence/absence of 5 mM histidine. Three replicates for each condition chosen were tested. Briefly, *M. morganii* DSM30164^T was grown in LB broth at 37 °C overnight and then reinoculated into 50 ml LB broth at a 1 % level in a shaker set at 200 rev/min, until the culture reached early exponential phase of growth ($OD_{600nm} = 0.3$), determined through growth curve analysis in LB. Then, cells were collected by centrifugation (10,000×*g*, 10 min, 4 °C), washed with PBS, and resuspended in 20 ml of the appropriate environment. Cells were exposed to each environment for 1 h at 37 °C,

without shaking, before RNA was extracted for gene expression analysis. All samples were checked for mRNA concentration and purity through the measure of the absorbance at 260 and 280 nm with a UV–Vis spectrophotometer (SmartSpec_ Plus; Bio-Rad), and for integrity through agarose gel electrophoresis. RNA was treated with DNaseI, Rnase-Free kit (Fermentas, Lituania) at 37 °C for 1 h, before the reaction was stopped with EDTA (50 mM) and incubation at 65 °C for 10 min. RNA was stored at −80 °C and subsequently used for cDNA synthesis.

RT-qPCR Experiments

Amplification, detection, and real-time analyses were performed using a CFX96 Real-Time PCR (Bio-Rad). SYBR Green (Bio-Rad) was used for detection of the amplified product. Sequence data for primer design were obtained from the Entrez database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The nucleotide sequences of primers used in this study for target genes *hdc* (histidine decarboxylase), *his*RS (histidyl-tRNA synthetase), and reference gene (16S rDNA) are listed in Table 1. Conditions for all PCRs were optimized with regard to *Taq* DNA polymerase, primer concentration, and annealing temperature. Normalized RNA was used to generate cDNA by reverse transcription using reagents supplied in the RevertAid First strand cDNA Synthesis Kit (Fermentas) in accordance with manufacturer's instructions. Two µl of cDNA was used as PCR template in a 15 µl reaction mixture containing 4.78 µl water, 0.72 µl primer mix (containing 0.3 µM of each primer), and 7.5 µl of the SsoFast_EvaGreen_Supermix (Bio-Rad). The following experimental run protocol was used: initial denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s, and annealing at 60 °C for 30 s. Specificity of RT-PCR products was documented performing a melting curve analysis which resulted in single product specific melting temperatures as follows: *hdc,* 81.5 °C, *his*RS, 86 °C, 16S rDNA 84 °C. For each target gene, the real-time-PCR efficiency was calculated according to the equation $E = 10^{(-1/\text{slope})}$ [26]. The level of gene expression was calculated according to the Expression Ratio Equation proposed by Pfaffl [24]. The significance of the results was analyzed by Student's *t* test with a two-tailed distribution. Differences with *P* values of 0.05 were considered significant.

Database Search and Sequence Comparison

The database searches were performed by using the basic local alignment tool (BLAST) program [2], from the National Center For Biotechnology Information BLAST website. Multiple sequence alignment and calculation of the phylogenetic relationships were performed using ClustalW [30].

Nucleotide Sequence Accession Number

The nucleotide sequences described here have deposited in the GenBank database under accession number KC771251.

Results

Sequence Analysis of the *hdc* Gene Cluster of *M. morganii*

Through a plasmid-DNA library, a *M. morganii* DNA region of 7,681 bp around the *hdc* was sequenced. Computer analysis of this region revealed, in addition to the *hdc* gene, the presence of other three open reading frames (ORFs), oriented in the same direction. We designated these four ORFs as *hdcT1, hdc, hdcT2*, and *hisRS*, respectively (Fig. 1a). The histidine decarboxylase gene, already described for a strain of *M. morganii* [32] is 1,137 bp long, with an initial start codon (ATG) and a stop codon (TAA) at the 3′ end, followed by a 8-base inverted repeat which could represent a potential transcription termination site. It encodes 377 amino acid residues with a calculated molecular mass of 42,900 Da. The catalytic residue lysine at position 232 confirms the PLP-dependent nature of the enzyme. The deduced amino acid sequence of *M. morganii* Hdc shares significant similarity (81–99 %) with those of *P. damselae, P. phosphoreum, E. aerogenens,* and *R. planticola.* As reported by other authors [18], this enzyme, distributed among Gram negative bacteria, seems evolved from a common ancestral gene (Fig. 1 b), if compared with Hdc from Gram positive bacteria. Also, consistent with the previous literature [28], *Photobacterium* strains appeared to be the most distant from the other enteric species.

The 1,362-bp ORF encoding a 453 amino acid (48,200 Da) protein, positioned at 50-bp upstream of *hdc* shows the most significant similarity to basic amino acid/polyamine transporter (antiporter) family. Particularly, *hdcT1* showed 82 % identity to a hypothetical protein of *K. oxytoca* (subsequently identified as a putative antiporter trough BLAST analysis), an amino acid permease of *E. aerogenens*, and 73 % identity with an histidine/histamine antiporter (HdcT) of *P. damselae*. Another putative histidine/histamine antiporter ORF, designed *hdcT2*, was located 206-bp downstream the *hdc* gene. This gene is 1,443 bp in length and encodes a 480 amino acid (51,700 Da) protein showing significant similarity to amino acid antiporters of *K. oxyt*oca (60 % identity), *P. damselae* (50 %), and to an amino acid permease of *E. aerogenes* (55 %). Amino acid sequence comparison between HdcT1 and HdcT2 of *M.*

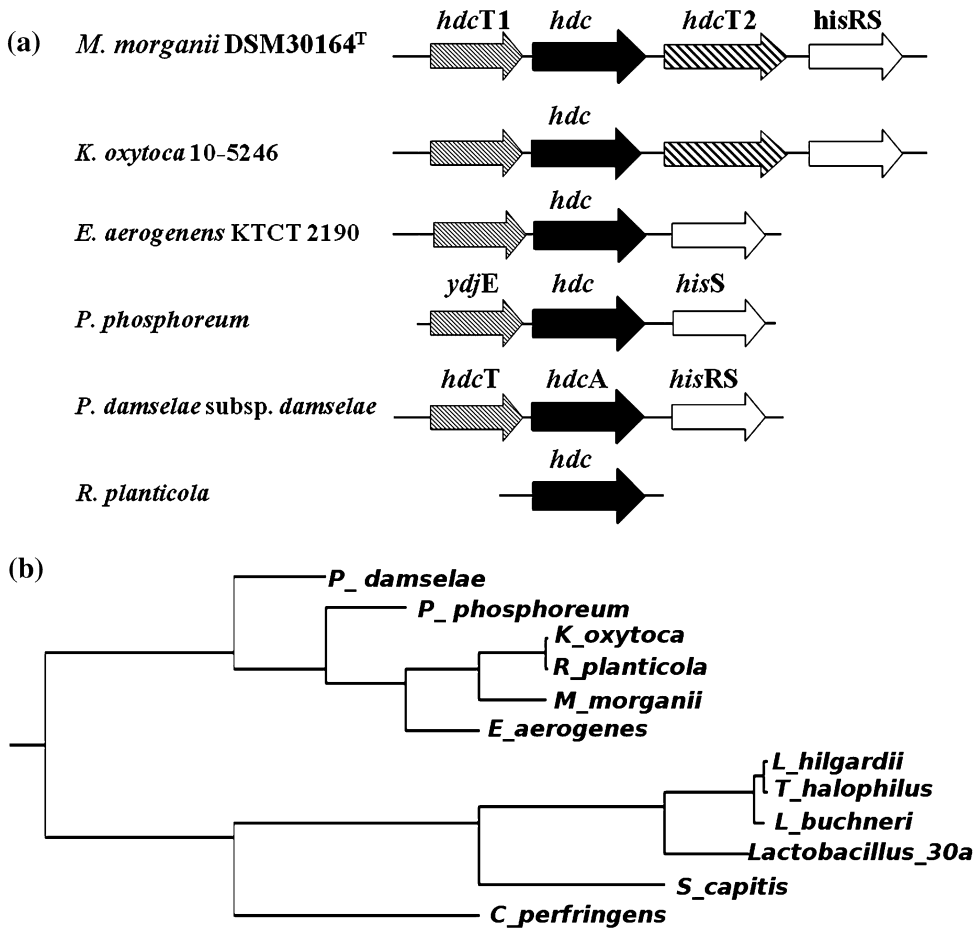

Fig. 1 **a** Schematic representation of the histidine decarboxylase cluster of *M. morganii* DSM30164^T, *K. oxytoca* 10-5246, *E. aerogenens* KTCT 2190 (CP002824), *P. damselae* (AB363972), *P. phosphoreum* (AY223843), and *R. planticola* (AAA25071). *Arrows* indicate ORFs. Genes coding for Hdc are represented in *black*, putative antiporters or amino acid permeases are hatched, while histidyl-tRNA synthetases are *white*. **b** Phylogenetic relationships of bacteria based on Hcd sequences. Distances between sequences were calculated by ClustalW software and dendrograms were drawn by the UPGMA method. Hdc sequences of Gram positive bacteria were included in the phylogenetic analysis (*Clostridium perfringens* J02880, *Staphylococcus capitis* AM283479, *Lactobacillus* 30a J02613, *Lactobacillus buchneri* AJ749838, *L. hilgardii* AY651779, and *Tetragenococcus halophilus* AB362339)

morganii and more characterized amino acid/amine antiporters (HdcC of *Lactobacillus buchneri*, HdcP of *Staphylococcus capitis*, and HdcT of *P. damselae*) identified several conserved residues typical of the histidine/histamine antiporter proteins (data not shown).

Downstream the *hdc*T2 gene, a 1,272 bp ORF designated *hisRS* with a coding capacity of 423 amino acid was found. The HisRS protein has a molecular mass of 48,400 Da and shows similarity to histidyl-tRNA synthetases (HisRS) from other Gram negative histamine producing bacteria, such as *K. oxytoca*, *E. aerogenes*, and *P. damselae*, (ID ranging from 81 to 78 %). Amino acid sequence comparison between HisRS of *M. morganii* and HisRS proteins of other Gram negative bacteria identified conserved motifs of class II amino acyl-tRNA synthetases (Fig. 2). Within the histamine producing Gram negative bacteria, only *K. oxytoca* shows an organization of the *hdc* gene cluster similar to that found for *M. morganii* (as deduced by analysis of the genome of *K. oxytoca* 10-5246, Accession AGDM 00000000), characterized by the occurrence of two histidine/histamine antiporters located upstream and downstream the *hdc* gene (Fig. 1).

Expression of *hdc* Gene in *E. coli*

To confirm its function, *hdc* gene was cloned into the plasmid pURI3, transformed into *E. coli*, and the Hdc presence verified by SDS-PAGE and affinity purification step (Fig. 3a). Enzyme activity of the recombinant *E. coli* pURI3hdc cells was negligible in the absence of IPTG. In the presence of IPTG, Hdc could be detected within 30 min of induction and increased for 4 h when induced at 37 °C, longer induction periods resulted in decreased activities. After purification, the ability of the Hdc protein to decarboxylate histidine was assayed. Figure 3b shows the histamine spot on TLC plates, indicating that the histidine decarboxylase is a functional protein.

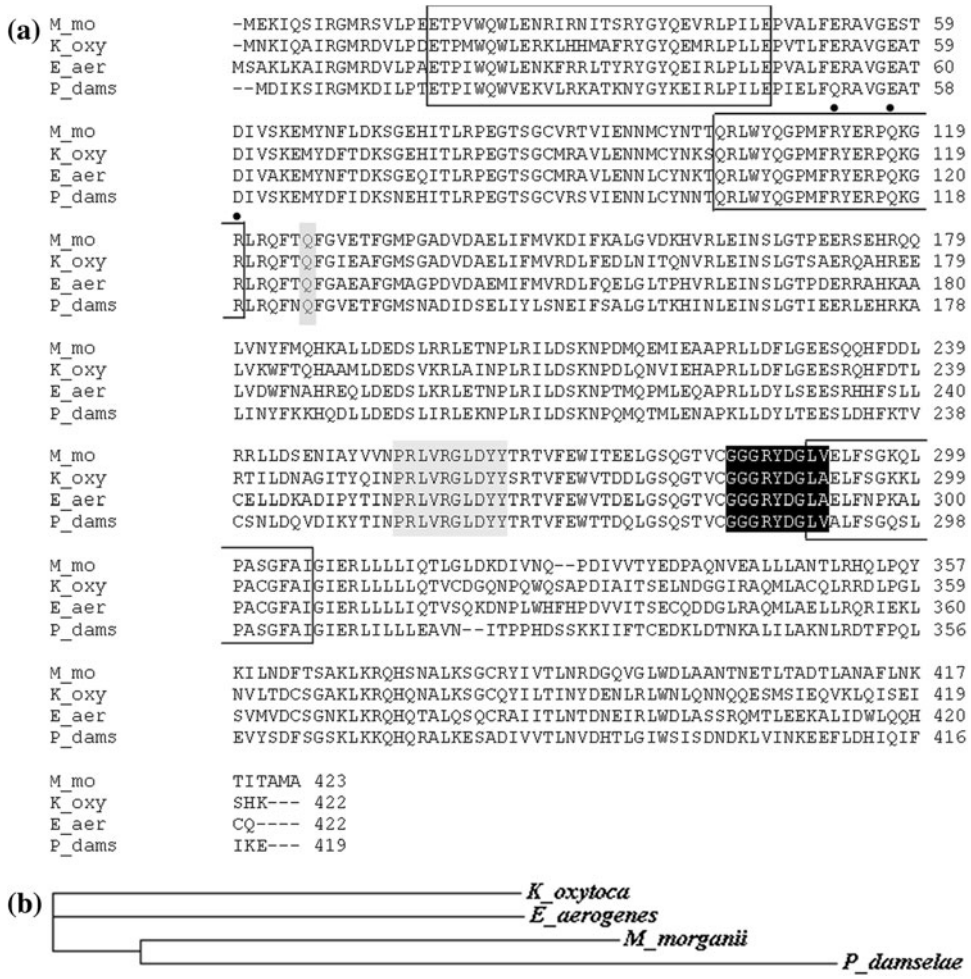

Fig. 2 **a** Alignment of the HisRS sequences of *M. morganii* DSM30164^T (M_mo), *K. oxytoca* 10-5246 (K_oxy), *E. aerogenens* KTCT 2190 (E_aer), and *P. damselae* (P_dams). Significantly conserved domains of class II aminoacyl-tRNA synthetase protein are *boxed*. *Grey* and *black boxes* indicate domains related to the interaction of histidine substrate with histidyl-tRNA synthetase in *E. coli*. *Black dots* indicate important residues for specificity of histidyl-tRNA synthetase to histidyl-tRNA molecules in *E. coli* [3, 15]. **b** Phylogenetic relationships of bacteria based on deduced amino acid sequences. Distances between sequences were calculated by ClustalW software and dendrograms were drawn by the neighbor-joining method

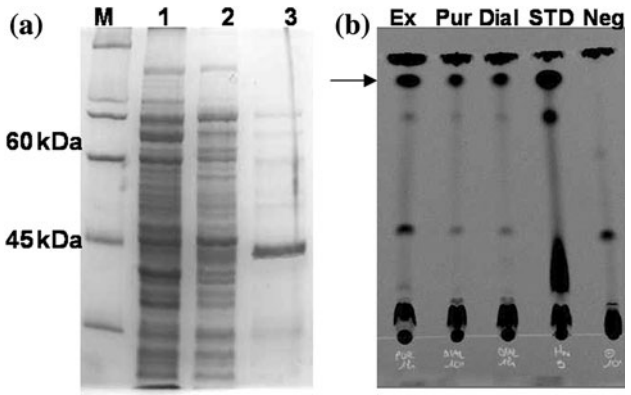

Fig. 3 **a** SDS gel of the purified Hdc protein. *Lanes: M marker*; *1 E. coli* pURI3 negative control; *2 E. coli* pURI3hdc; *3* purified Hdc. **b** Histamine detection by TLC after dansylation: cell extract (*Ex*), purified (*Pur*) and dialized (*Dial*) protein, histamine standard (*STD*), and negative control (*Neg*, *E. coli* pURI3 cell extract). The *arrow* indicates the histamine spot

Expression of the Histidine Decarboxylase-Related Genes

The expression level of *hdc* and *his*RS in response to nutrient concentration, histidine content, and pH were detected and are depicted in Fig. 4. For each gene, amplifications were performed on three independent RNA samples from each condition. For 16S rDNA, chosen as endogenous control, no significant differences in expression level were detected, regardless the condition tested. All comparison were made between values obtained from amplification of RNA derived from the different environmental conditions applied, relative to those obtained from amplifications of RNA from control conditions (LB medium, 37 °C, pH 7.0), and normalized using the 16S rDNA endogenous control. The expression of *hdc* and *hisRS* genes was induced under the conditions of low pH, as reported in Fig. 4. Statistically significant differences

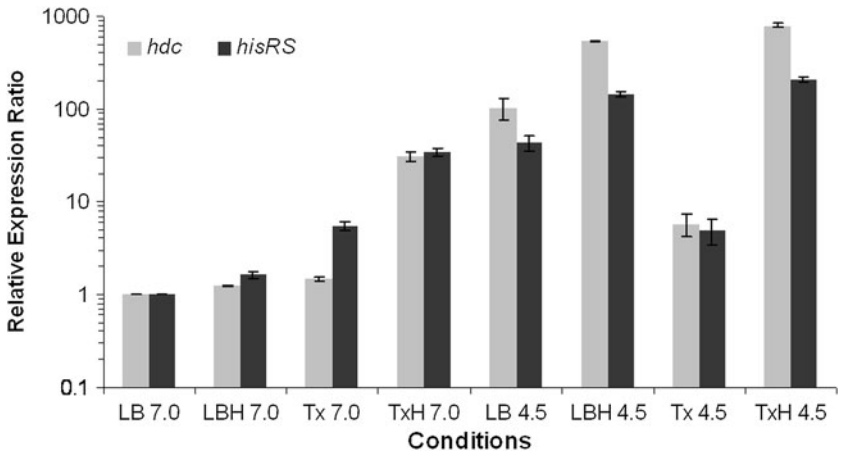

Fig. 4 Relative gene expression of *hdc* and *hisRS* genes, normalized to the level of 16S rDNA, of *M. morganii* under different culture conditions: LB-rich medium (LB), with histidine (LBH) at pH 7.0 and 4.5 (LB/LBH 7.0 and LB/LBH 4.5, respectively); Tx minimal medium (Tx), with histidine (TxH) at pH 7.0 and 4.5 (Tx/TxH 7.0 and Tx/TxH 4.5, respectively)

($P < 0.05$) were detected after exposure to LB medium at pH 4.5; this condition induced an increase of 100- and 40-fold, of *hdc* and *his*RS expression, respectively, as compared to LB at pH 7.0 (Relative Expression, RE 103.82 and 43.71). In the same acidic condition, the presence of 5 mM histidine further increased the expression of these genes 5- and 3-fold (RE 540.92 and 144.33). The greatest expression of *hdc* and *hisRS* was obtained when *M. morganii* cells were exposed to minimal medium Tx, at pH 4.5 and in the presence of histidine ($P < 0.05$). In this case, the relative expression increased 800- and 210-fold, respectively. In the other conditions tested, low values of expression were detected.

Discussion

We isolated and sequenced the histidine decarboxylase gene cluster of *M. morganii* DSM30146^T, which was composed by four ORFs, including a putative histidine/histamine antiporter, *hdcT1*, the histidine decarboxylase *hdc*, another putative histidine/histamine antiporter, designed *hdcT2*, and a putative histidyl-tRNA synthetases (*hisRS*), oriented in the same direction. This organization, particularly the presence of genes codifying an amino acid permease/antiporter and an histidyl t-RNA synthetase in flanking regions of *hdc* gene was comparable with the gene cluster of other known Gram negative bacteria, *P. damselae, P. phosphoreum, E. aerogenes* [18]. Thus, the mechanism of histamine formation by *M. morganii* seems involve two specific enzymes, a putative antiporter, which takes the histidine from outside and excretes histamine from the cell, and a histidine decarboxylase which catalyzes the reaction of decarboxylation. The histidyl-tRNA synthetase gene within the cluster might play a regulatory role in attenuation control, as reported for other Gram negative bacteria [11]. The presence of an additional histidine/histamine antiporter, detected downstream the *hdc* gene, indicates a gene organization similar to that of *K. oxytoca* 10-5246. However, the reason for the presence of two putative amino acid antiporters remains to be elucidated.

RT-qPCR experiments revealed the *hdc* and *hisRS* genes were highly induced under acidic and histidine-rich conditions. A higher expression of the *hdc* and *hisRS* genes was obtained combining the above reported conditions with a minimal medium. We could hypothesize that the *hdc* cluster in *M. morganii* is particularly upregulated if, in addition to the primary stimulus (low pH), the substrate for the decarboxylase is present (high histidine level) and the product of the reaction is absent (low histamine level).

Bacterial amino acid decarboxylases are hypothesized to play a role in controlling pH to countermeasure the acidity resulting from fermentations [18]. This mechanism is generally considered related to the decreasing of intercellular proton concentration through the decarboxylation reaction and to the improvement of the microenvironment by excretion of a basic molecule, the amine. According to the models proposed, accumulation of histamine by *M. morganii* may confer resistance to acid stress developed through consuming intracellular protons via the decarboxylation reaction.

This is the first report of cloning and sequencing of histidine decarboxylase gene cluster in *M. morganii*. Further studies are necessary to better understand the organization of this gene cluster. Our preliminary results indicate that *hdc* and *hisRS*, that seem regulated in the same way, are not part of a same operon. The recent availability of the genome sequence of *M. morganii* strain KT deposited in public database (Bioproject: PRJNA 78681, Accession: ALJX00000000) permitted us to verify the presence of unique *hdc* and *his*RS genes along the genome. The reasons for induction of *hisRS* expression under high concentration of extracellular histidine and the real action of the transcriptional terminator downstream the *hdc* gene are to be in depth investigated, by mean of further transcriptional and cloning experiments.

Acknowledgments This work was supported by grants AGL2008-01052, AGL2011-22745, and Consolider INGENIO 2010 CSD2007-00063 FUN-C-FOOD (CICYT), S2009/AGR-1469 (ALIBIRD) (CAM), and RM.2012-00004 (INIA). We are grateful to J. Barcenilla and M. V. Santamaria.

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