Identification of the Enterococcus faecalis Tyrosine Decarboxylase Operon Involved in Tyramine Production

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Screening of a library of Enterococcus faecalis insertional mutants allowed isolation of a mutant affected in tyramine production. The growth of this mutant was similar to that of the wild-type E. faecalis JH2-2 strain in Maijala broth, whereas high-performance liquid chromatography analyses showed that tyramine production, which reached 1,000 μg ml⁻¹ for the wild-type strain, was completely abolished. Genetic analysis of the insertion locus revealed a gene encoding a decarboxylase with similarity to eukaryotic tyrosine decarboxylases. Sequence analysis revealed a pyridoxal phosphate binding site, indicating that this enzyme belongs to the family of amino acid decarboxylases using this cofactor. Reverse transcription-PCR analyses demonstrated that the gene (tdc) encoding the putative tyrosine decarboxylase of E. faecalis JH2-2 is cotranscribed with the downstream gene encoding a putative tyrosine-tyramine antipporter and with the upstream tyrosyl-tRNA synthetase gene. This study is the first description of a tyrosine decarboxylase gene in prokaryotes.

Biogenic amines in food result mainly from microbial activity due to amino acid decarboxylation (16, 49). Histamine and tyramine have been the most studied biogenic amines due to the toxicological effects derived from their vasoactive and psychoactive properties. Histamine has been recognized as the causative agent of scromboid poisoning (histamine intoxication), whereas tyramine has been related to food-induced migraines and hypertensive crisis (39). Various tyramine concentrations have been found in many foods, including cheeses, drinks, and meat and fish products (30, 44, 45), and a dose of only 6 mg total tyramine intake may be dangerous for patients under antidepressive treatment who are receiving monoamine oxidase inhibitors (42). The formation of tyramine in foods depends on the concentration of free tyrosine and the presence of microorganisms having tyrosine decarboxylase activity. Many microorganisms could be implicated in tyramine production. For example, some bacteria belonging to the genera Enterococcus, Carnobacterium, and Lactobacillus have been found to be tyramine producers (5, 31, 35). However, while tyrosine decarboxylase enzymes have been well characterized in eukaryotes, for example, in parsley (previously called Streptococcus carnose) (4) and for L. brevis (32), little is known about tyrosine decarboxylase in prokaryotes.

The aim of this study was to isolate and characterize the gene encoding tyrosine decarboxylase in E. faecalis JH2-2. For this purpose, a library of E. faecalis JH2-2 insertional mutants was screened for mutations affecting tyramine production. Isolation and characterization of a tyrosine decarboxylase mutant allowed the identification and genetic analysis of the tyrosine decarboxylase determinants of E. faecalis JH2-2.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The present study was performed using the E. faecalis strain JH2-2 (57), which was obtained from the parental strain JH2 (22). E. faecalis was grown at 37°C in M17 medium supplemented with 0.5% glucose (GM17) (50). When necessary, the antibiotics erythromycin and chloramphenicol were used at 150 and 20 μg ml⁻¹, respectively. Modified Maijala decarboxylation broth (29) containing 2 g of tyrosine per liter was used for screening tyrosine decarboxylase mutants in microtiter plates. Escherichia coli strain EC101 containing the repA gene for replication of pWV01-type plasmids (26) was grown in Luria-Bertani medium (40) with 100 μg of tetracycline or with 150 μg of erythromycin ml⁻¹ to maintain the pOR119 plasmid and derivatives (26). Plasmid pG/ho (previously named pVE6007) (28), encoding a thermosensitive RepA protein, was maintained in E. coli TGI (Strategene) at 30°C with 10 μg of chloramphenicol ml⁻¹.

Isolation of insertional mutants of E. faecalis JH2-2. The library of insertional mutants of E. faecalis JH2-2 used in this study was constructed with the strategy described by Law et al. (26) for Lactococcus lactis. Briefly, 200-bp to 1.5-kb chromosomal DNA fragments generated by partial digestion with endonuclease Alul were cloned into the pOR119 plasmid (pWV01-derived Ori’ RepA’.) using the RepA helper E. coli strain EC101 (26) to obtain a bank of approximately 37,200 recombinant plasmids. A mixture of these recombinant plasmids was then transferred into E. faecalis JH2-2, which had previously received the pWV01-derived Ori’ RepA’ pg’ host3 plasmid (28). Clones were grown at 30°C in GM17 medium containing erythromycin and chloramphenicol (the thermosen-
sitive Rep\textsuperscript{AT} protein is active at 30°C and allowed replication of the pG\textsuperscript{+} host3 and pORI19 recombinant plasmids. The cells were then transferred to GM17 containing erythromycin, and the incubation temperature was shifted to 42°C to inactivate the Rep\textsuperscript{AT} protein and consequently occasion the loss of pG\textsuperscript{+} host3 and the integration of the pORI19 recombinant plasmid by homologous recombination.

Excision and curing of integrated plasmids. For sequencing experiments, excision of the plasmids integrated in mutants 16G10 and 16G12 was performed by transformation with pG\textsuperscript{+} host3 and selection of the transformants at 30°C on GM17 plates with chloramphenicol and erythromycin. At this permissive temperature, the active Rep\textsuperscript{AT} protein allows the replication of both plasmids and thus favors the excision of the pORI19 recombinant plasmid. For curing of the integrated plasmid from mutant 16G10, transformants were selected at 30°C on GM17 plates with chloramphenicol, grown for 100 generations at 30°C on GM17 broth containing chloramphenicol, and then grown for 1 h at 30°C in GM17 without antibiotics and transferred at 42°C for 3 h before being plated in the same medium and incubated at 42°C. One of the 11% of the isolated clones was found to be sensitive to erythromycin. It was tested for its tyrosine decarboxylase activity using chromosomal DNA as a template; primers F4S1 and F3S2; and the integration of the pORI19 recombinant plasmid by homologous recombination.

DNA sequencing. DNA sequencing was performed on PCR fragments purified with the QiAquick kit (Qiagen) and using the dideoxy chain termination method (41) with the ABI prism sequencing system (PE Biosystems). To eliminate the eventual effects of Taq DNA polymerase mistakes, a mix of three independent amplifications was sequenced.

**Tyramine quantification by HPLC.** The growth and tyramine production of *E. faecalis* HJ-2 and the 16G10 mutant were studied in Majala broth as follows. Majala broth was inoculated at 10\textsuperscript{6} CFU ml\textsuperscript{−1} with overnight precultures of *E. faecalis* HJ-2 and the 16G10 mutant in GM17. The cells were enumerated on GM17 agar for 24 h and simultaneously analyzed by HPLC for tyramine production. For this, cultures were centrifuged at 8,000 × g for 10 min, and the resulting supernatants were dansylated as previously described by Eerola and Hinkannen (13). Dansylated samples were analyzed on a C\textsubscript{18} ODS2 Equisorb column (4.6 by 250 mm; particle size, 5 μm) using a Constametric apparatus (Ldc; Milton Roy). The elution gradient began with 45% 0.1 M ammonium acetate and 55% acetonitrile and ended at 10% 0.1 M ammonium acetate and 90% acetonitrile in 18 min at 40°C under a flow rate of 1 ml min\textsuperscript{−1}. Dansylated tyramine was detected by UV absorption at 254 nm and was quantified using a calibration curve.

**Analysis of RNAs by Northern blotting and reverse transcription (RT)-PCR experiments.** Total RNA of *E. faecalis* HJ-2 was isolated from exponentially growing cells in Majala broth by using the RNeasy Midi kit (Qiagen). Northern blots of exactly 10 μg of electrophoresed RNA were prepared by using Hybond-N\textsuperscript{+} membranes and standard procedures (40). The sizes of the transcripts were estimated by comparing band mobility with RNA ladder standards (0.56 to 9.4 kb) (Amersham International, Little Chalfont, United Kingdom). Membrane-bound nucleic acids were hybridized with single-stranded labeled probes as described for Southern blot hybridization. Preparation of the single-stranded labeled probes was as follows. First, a DNA fragment was amplified by PCR using chromosomal DNA of *E. faecalis* HJ-2 and primers F5S1 and F5S2 (Table 1) for ORF1, primers F5S1 and F5S2 for the tdc gene, and F8S1 and F8S2 for ORF3. Then, the probes were synthesized by elongation of the oligonucleotide F5S2, F5S2, or F8S2 using Taq DNA polymerase; 2 μM (each) dCTP, dGTP, and dTTP; and 2 μCi of [\textsuperscript{32}P]dATP.

**TABLE 1. Primers used for PCR and sequencing experiments**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Orientation</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5S1</td>
<td>5′-GTGTTGTAATCGTTTGTTCG-3′</td>
<td>+</td>
<td>Upstream of ORF\textsuperscript{1}</td>
</tr>
<tr>
<td>F5S2</td>
<td>5′-GTTGATATGCGCCCATGATC-3′</td>
<td>+</td>
<td>pORI19 (620–689)</td>
</tr>
<tr>
<td>F6S1</td>
<td>5′-GGCATTCTCCTTACAGAATTCAC-3′</td>
<td>+</td>
<td>ORF1 (521–543)</td>
</tr>
<tr>
<td>F6S2</td>
<td>5′-CATCGAAGATTGGTGGATGAC-3′</td>
<td>+</td>
<td>ORF1 (1223–1244)</td>
</tr>
<tr>
<td>F7S1</td>
<td>5′-GCTATAATGTTAAACGGTGAC-3′</td>
<td>+</td>
<td>ORF1 (1175–1195)</td>
</tr>
<tr>
<td>F7S2</td>
<td>5′-CTAATCGCATCAACAGTCG-3′</td>
<td>+</td>
<td>ORF2 (1692–1710)</td>
</tr>
<tr>
<td>F8S1</td>
<td>5′-CTTAAACACATTTTGTTGGG-3′</td>
<td>+</td>
<td>ORF2 (1641–1662)</td>
</tr>
<tr>
<td>F8S2</td>
<td>5′-GTGCTGGAATTGGTCTGCC-3′</td>
<td>+</td>
<td>ORF2 (2262–2283)</td>
</tr>
<tr>
<td>F9S1</td>
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<td>+</td>
<td>ORF2 (2208–2223)</td>
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<tr>
<td>F9S2</td>
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<td>ORF2 (2840–2864)</td>
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<tr>
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<td>5′-CACGCTGTTGATCGTTATC-3′</td>
<td>+</td>
<td>ORF2 (2800–2820)</td>
</tr>
<tr>
<td>F10S2</td>
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<td>+</td>
<td>ORF3 (3501–3519)</td>
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<tr>
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<td>+</td>
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<tr>
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<td>+</td>
<td>ORF3 (3956–3974)</td>
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<td>5′-GGTTAATGCTTACTGCCTGCC-3′</td>
<td>+</td>
<td>ORF3 (3850–3824)</td>
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<tr>
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<td>5′-GGAAATATGAGCACAACACG-3′</td>
<td>+</td>
<td>ORF3 (4425–4445)</td>
</tr>
<tr>
<td>F9S1</td>
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<td>+</td>
<td>ORF3 (4195–4212)</td>
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<tr>
<td>F9S2</td>
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<td>+</td>
<td>ORF3 (4735–4753)</td>
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<tr>
<td>F10S1</td>
<td>5′-CTCGTCTGGCTTACGAC-3′</td>
<td>+</td>
<td>ORF3 (4574–4591)</td>
</tr>
<tr>
<td>F10S2</td>
<td>5′-CATCAGGTTGAAAGGCAC-3′</td>
<td>+</td>
<td>Downstream of ORF3\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}+ , primers directed towards the 3′ ends of ORFs. – , primers oriented in the opposite direction.

\textsuperscript{b}Location is given related to the nucleotide sequence deposited under accession number AF354231.

\textsuperscript{c}5′ ends of primers F8S1 and F8S2 are located 25 bp upstream of the first nucleotide and 36 bp downstream of the last nucleotide from the sequence deposited under accession number AF354231, respectively.

\textsuperscript{d}Primer F6S2 is located between ORF2 and ORF3.

**Southern blot analysis.** For Southern blot analysis, chromosomal DNA of *E. faecalis* was isolated from a 3-ml culture of strain JH2-2 as follows. Cells in stationary phase were harvested by centrifugation, resuspended in 0.5 ml of lysozyme (5 mg ml\textsuperscript{−1}) and incubated for 20 min at 37°C. Then, 20 μl of protease K (2%) and 25 μl of a 10% sodium dodecyl sulfate (SDS) solution were added, and lysis was obtained after incubation at 60°C. DNA was then prepared as described by Sambrook et al. (40) and resuspended in 10 mM Tris-HCl, 1 mM EDTA (pH 7.5) containing 500 μg of RNAse ml\textsuperscript{−1}.

**Southern blot hybridization.** For Southern blot analysis, chromosomal DNA digested with restriction enzymes was electrophoresed on a 0.8% agarose gel and transferred to a Hybond-N\textsuperscript{+} membrane (Amersham) by capillary transfer as recommended by the membrane manufacturer. Membrane-bound nucleic acids were hybridized at a temperature of 55°C in 1 M sodium phosphate buffer (pH 7.0) containing 5% SDS with a [\textsuperscript{32}P]-labeled probe. After hybridization, the membranes were washed twice successively in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% SDS (10 min) and twice in 0.5× SSC-0.1% SDS (10 min) at 55°C and exposed to a storage phosphor screen (Packard Instrument Company, Canberra, Australia) for 5 h.

Preparation of the double-stranded labeled probe was carried out by PCR using *E. faecalis* chromosomal DNA as a template; primers F5S1 and F5S2; Taq DNA polymerase; 2 μM (each) dCTP, dGTP, and dTTP; and 2 μCi of [\textsuperscript{32}P]dATP.

**Primer F5S2 is located between:**
cDNAs were then purified with the QIAquick kit (Qiagen). RT-PCRs were performed using purified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech). Amplifications were performed for 30 cycles consisting of 94°C for 30 s, 55°C for 30 s, and 72°C for 210 s, and the samples were analyzed on a 0.8% agarose gel in TAE (40 mM Tris-acetate [pH 8.0], 1 mM EDTA) buffer. The absence of contaminating genomic DNA was controlled by non-reverse-transcribed PCR performed under the same conditions, except that avian myeloblastosis virus reverse transcriptase was replaced by H2O.

**General molecular methods.** Restriction endonucleases were obtained from Amersham International. PCRs were carried out with 5 μg of chromosomal DNA of *E. faecalis* JH2-2 and 20 pmol of primers using Ready To Go PCR beads (Pharmacia Biotech). *E. coli* and *E. faecalis* were transformed using a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.) as described by Dower et al. (11) and Holo and Nes (19), respectively. DNA and amino acid sequences were analyzed using the Mac Vector program (Kodak Scientific Imaging Systems), database searches were performed with the BLAST program (1), and amino acid sequence alignments were carried out with Clustal W (51). Other standard techniques were carried out as described by Sambrook et al. (40).

**Nucleotide sequence accession number.** The DNA sequence of the tyrosine decarboxylase operon determined in this paper has been submitted to the EMBL, GenBank, and DDBJ nucleotide databases under accession number AF354231.

### RESULTS

**Isolation of *E. faecalis* JH2-2 tyrosine decarboxylase mutants.** A library of 9,600 insertional mutants of *E. faecalis* JH2-2 constructed with the strategy described in Materials and Methods was screened for tyrosine decarboxylase mutants. Cultures of individual colonies were grown at 37°C in 96-well microtiter plates in GM17 broth containing erythromycin and replica plated in Maijala broth. Tyramine producer colonies were transformed using purified *E. coli* (Pharmacia Biotech). *E. faecalis* JH2-2 and 20 pmol of primers using Ready To Go PCR beads (Pharmacia Biotech). Amplified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech). Amplified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech). Amplified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech). Amplified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech). Amplified cDNA as a template, 20 pmol of primers, and Ready To Go PCR beads (Pharmacia Biotech).

**Genomic characterization of the *E. faecalis* tyrosine decarboxylase mutants.** Integrated plasmids in mutants 16G10 and 16G12 were excised as described in Materials and Methods. DNA sequence analysis revealed that plasmids excised from both mutants contained identical 664-bp DNA inserts, so further characterizations were performed only with the 16G10 mutant. Comparison with the *E. faecalis* (V583) genomic sequence (available at http://www.tigr.org) revealed that this 664-bp segment corresponded to a central region of a 1,860-bp open reading frame (ORF), thereafter named tyrosine decarboxylase (*tdc*).

Oligonucleotides designed from the *E. faecalis* V583 genome (Table 1) were used to verify the integration locus of the 16G10 mutant and to sequence the corresponding gene and adjacent ORF from *E. faecalis* JH2-2. A Southern blot of total chromosomal DNA extracted from the 16G10 mutant and from the wild-type JH2-2 strain and digested with EcoRI or *Kpn*I plus SacI gave a 5,094-bp nucleotide sequence of the *E. faecalis* JH2-2 *tdc* gene and the adjacent ORF sequence was determined from PCR products and deposited in the EMBL, GenBank, and DDBJ nucleotide databases.

**Sequence analysis.** Observation of the nucleotide sequence immediately upstream of the *E. faecalis* JH2-2 *tdc* ORF gave evidence for the presence of a ribosome binding site sequence (GGAGG) located 9 bp upstream of the initiation codon (ATG). Translation of the entire ORF revealed that it encodes a 620-amino-acid protein of 70.0 kDa having homologies with decarboxylases (Fig. 2). The best homology scores were observed with the probable glutamate decarboxylase of *Vibrio cholerae* (24% identity; 40% similarity [17]). Furthermore, the tyrosine decarboxylase sequence of *E. faecalis* JH2-2 contained the consensus pattern observed for pyridoxal phosphate-dependent decarboxylases (also known as group II decarboxylases, S-[LIVMFYW]-X(5)-K-[LIVMFYWG]-[LIVMFYWG]-X(3)-[LIVMFYW]-X-[CA]-X(2)-[LIVMFYWG]-X(2)-[RK] (http://www.expasy.ch/cgi-bin/nicedoc.pl?PD0C00329), where lysine (K) is the attachment site for the cofactor pyridoxal phosphate. However, in our case, the ultimate amino acid of the consensus pattern would not be arginine (R) or lysine but glutamine (Q) (Fig. 2). Furthermore, *E. faecalis* JH2-2 Tdc has 20% identity with the tyrosine decarboxylase II sequence from *parsley* (*P. crispum*), including the VHVDAAAYmotif (Fig. 2) particularly conserved in pyridoxal phosphate-dependent decarboxylases (24). Upstream of the *tdc* gene, we can find an ORF (*ORF1*) corresponding to a 47.3-kDa protein having high homology with tyrosyl tRNA synthetases (54% identity and 72% similarity with the tyrosyl tRNA synthetase of *Bacillus subtilis* [18]). Tyrosyl tRNA synthetases are aminooacyl-tRNA synthetases of class I characterized by the HIGH and KMSKS
pyridoxal phosphate attachment site. The boxes represent the VHVDAAY motif and the pyridoxal phosphate attachment site. An underlined number in a sequence indicates the number of amino acids omitted at that position to save space.

**Growth and tyramine quantification of E. faecalis JH2-2 and the 16G10 mutant.** The growth of E. faecalis JH2-2 and the 16G10 mutant in Maijala broth followed similar patterns (Fig. 3). Bacterial populations increased from 10^7 CFU ml^-1 to 10^9 CFU ml^-1 in 24 h. Whereas the tyramine production of E. faecalis JH2-2 rose to reach approximately 1,000 μg ml^-1 in 24 h and the Maijala broth turned purple, no tyramine production was detected by HPLC for the 16G10 mutant, and the Maijala broth remained green (Fig. 3). To show that the tyramine production defect in mutant 16G10 was due to the plasmid insertion and not to an independent genomic mutation, the 16G10 mutant was cured from its pORI19-recombinant plasmid as described in Materials and Methods. This resulted in the restoration of tyramine production similar to that of the wild-type strain (data not shown).

**Transcriptional analysis.** Northern blot hybridization of RNA extracted from exponentially growing cells in Maijala broth with the single-stranded F2S2-F2S1 probe (tyrosyl tRNA synthetase gene) revealed a 1.4-kb mRNA (Fig. 4A). The size of this mRNA corresponded to the transcription of the tyrosyl tRNA synthetase gene alone, and the 3′ extremity of the transcript agreed with the presence of an inverted repeat 100 bp downstream of ORF1 (ΔG = −29.6 kcal/mol) which could act as a Rho-independent terminator. mRNAs corresponding to the tdc gene and to ORF3 were not visualized when the single-stranded probes F5S2-F5S1 or F8S2-F8S1 were used (data not shown), suggesting that these genes are less transcribed or that their corresponding transcripts are less stable.

RT-PCR experiments confirmed the transcription of the tyrosyl tRNA synthetase gene (Fig. 4B, lane 1) and provided evidence for the transcription of the tyrosine decarboxylase gene and the putative amino acid antiporter gene (Fig. 4B, lanes 3, 5, 7, and 9). When the cDNA from RT with primer F8S2 was amplified using primers F6S1 and F8S2, a large amount of amplimer was obtained (Fig. 4B, lane 9), showing that the tdc gene and the putative amino acid antiporter (ORF3) are cotranscribed. Moreover, observation of PCR products with primers F3S1 and F5S2 on cDNA from RT with F5S2 and with primers F3S1 and F8S2 on cDNA from RT with F8S2 (Fig. 4B, lanes 3 and 7, respectively), indicated that the tdc gene is also cotranscribed with the tyrosyl tRNA synthetase gene and that the three genes are cotranscribed. The existence of a cotranscription between the tyrosyl tRNA synthetase gene and genes encoding the tyrosine decarboxylase and the putative amino acid antiporter could be explained by an incomplete action of the putative Rho-independent terminator located between ORF1 and tdc.

**DISCUSSION**

In order to identify the gene encoding tyrosine decarboxylase in E. faecalis, a library of E. faecalis JH2-2 insertional mutants was screened for mutations affecting tyramine production. Two mutants unable to produce tyramine were isolated, and their genetic characterization revealed a unique integration locus corresponding to the central region of a 1,860-bp ORF (tdc). Comparison of tyramine production during the growth of the wild-type E. faecalis JH2-2 strain and of one mutant (16G10) confirmed the absence of tyramine production for the mutant and showed that the wild-type E. faecalis JH2-2 strain is able to produce up to 1,000 μg of tyramine ml^-1 after 24 h of culture in Maijala broth. The amount of tyramine produced by the wild-type strain E. faecalis JH2-2 is in agreement with the results obtained by Bover-Cid and Holzapfel (5), who found values from 601 to 4,986 μg ml^-1 for different E. faecalis strains. In the wild-type strain, tyramine production seems to be correlated with growth and may protect the bacteria from acidification.

Genetic characterization of the integration locus revealed that the 1,860-bp ORF encoded a decarboxylase (tyrosine decarboxylase) which had homology with amino acid decarboxylases from prokaryotes and tyrosine decarboxylase from parsley (P. crispum) (24). Furthermore, the presence of a motif corresponding to the attachment site of the pyridoxal phosphate.
Phosphate cofactor showed that the *E. faecalis* tyrosine decarboxylase belongs to the group of pyridoxal phosphate-dependent decarboxylases, which includes decarboxylases from eukaryotes and prokaryotes (21). To our knowledge, this study is the first description of a tyrosine decarboxylase gene in prokaryotes.

In *Salmonella enterica* serovar Typhimurium and *E. coli*, tyrosine is transported by a specific permease (a tyrP gene product) (55) and by the general aromatic amino acid transport system (an aroP gene product) (2, 6). Here, transcriptional analyses revealed that the tyrosine decarboxylase gene is cotranscribed with a gene encoding a potential amino acid antiporter, the latter probably being involved in tyrosine internalization. In *E. coli*, the lysine-cadaverine antiport cadB gene and the lysine decarboxylase cadA gene belong to the same operon, cadBA, and are cotranscribed under the control of the promoter Pcad (37). Recently, in *Listeria monocytogenes* and in *E. coli*, the glutamate decarboxylase gene, gadB, was also shown to be cotranscribed with gadC, a gene encoding a potential glutamate/γ-aminobutyrate antiporter (the γ-aminobutyrate is the product of glutamate decarboxylation) (8, 10). In *Lactococcus lactis* subspecies *lactis*, arginine is transported by an antiport system with ornithine (12), and in *E. coli*, the potE gene encodes a putrescine-ornithine antiporter (23). An antiport histidine-histamine has also been described in *Lactobacillus buchneri* (33). Similarly, in *E. faecalis*, the amino acid transporter encoded by the gene cotranscribed with tdc could be a tyrosine-tyramine antiporter.

The first tyrosine decarboxylase genetic sequence described here for a prokaryote organism (*E. faecalis*) could have an important impact. Indeed, it could be used to design primers that will allow fast and easy detection of tyramine producer strains in foods. Le Jeune et al. (27) compared the nucleotide sequences of histidine decarboxylase genes (*hdcA*) from *Lactobacillus* sp. strain 30A (52) and *Clostridium perfringens* (53) and compared the amino acid sequences of these histidine decarboxylases with those from *L. buchneri* (20) and *Micrococcus* (54). As alignment studies showed a high degree of relatedness among the *hdcA* gene products of gram-positive bacteria, these authors proposed PCR primers and genomic probes for identification of histidine-decarboxylating bacteria and tested them in numerous bacteria. Then, the primers were

![FIG. 4. Transcriptional analysis. (A) Northern blot hybridization of RNA isolated from exponentially growing *E. faecalis* JH2-2 cells in Maijala broth. Hybridization was performed with the F2S1-F2S2 single-stranded labeled probe. The size of the transcript was estimated by comparing the band mobility with those of standards in an RNA ladder (0.56 to 9.4 kb) (Amersham International). (B) RT-PCR assays conducted on mRNA isolated from exponentially growing *E. faecalis* JH2-2 cells in Maijala broth. RTs were performed with oligonucleotides F2S2 for lanes 1 and 2, F5S2 for lanes 3 to 6, and F8S2 for lanes 7 to 10. To ensure the absence of genomic DNA, negative controls were performed without reverse transcriptase (even lanes). PCRs were performed with primers F2S1 and F2S2 (lanes 1 and 2), F3S1 and F5S2 (lanes 3 and 4), F5S1 and F5S2 (lanes 5 and 6), F3S1 and F8S2 (lanes 7 and 8), or F6S1 and F8S2 (lanes 9 and 10). Lanes M contain DNA fragments of the molecular weight marker Smartladder (Euorgenec).](http://aem.asm.org/...
used to study efficiently the frequency and the distribution of histidine-decarboxylating bacteria in wines produced in south-west France (9). When more prokaryotic tyrosine decarboxylase genes are characterized, it should be possible to design primers in conserved regions for rapid detection of tyrosine-decarboxylating bacteria in foods. In this way, it may be possible in the future to prevent the toxicological effect of high tyramine consumption in food.

ACKNOWLEDGMENTS

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REFERENCES


