Coagulase-negative staphylococci (CNS) are often present in food-related samples and especially in fermented products (cheese or dry sausage) (4, 19, 23, 39). They are considered positive flora involved in the development of organoleptic characteristics of end products. Some strains are even used as starters (11, 41). However, although CNS are generally recognized as safe microorganisms, questions regarding presumption of safety have been raised for some CNS species which are investigated. The main risk factors identified correspond to the ability to produce biogenic amines (BA) (33, 34, 43, 44) because of the toxicological implication of these molecules in food products (cheese or dry sausage) (4, 19, 23, 39). They are considered responsible for BA formation have been extensively studied in different bacterial groups and especially in lactic acid bacteria (10, 14, 15, 26, 29, 30, 32, 33). Little work has been done on the different bacterial groups and especially in lactic acid bacteria.

Histamine and tyramine, the main studied amines, can lead to more or less severe intoxications when ingested in great quantities (27) or by people with some catabolic deficiency (9). Histamine and tyramine effect (21, 31). Most studies concerning biogenic amine production by staphylococci have been performed with fermented meat products (6, 33, 34, 35, 43). Other authors identified CNS strains that produced biogenic amines in other foodstuffs and especially fish-related samples (18, 22). Biogenic amines (BA) are produced in food matrices containing free amino acids via intracellular bacterial catabolic pathways that consist of a decarboxylase and a transporter responsible for the uptake of the amino acid and the excretion of the amine. The pathways convert histidine to histamine, tyrosine to tyramine, ornithine to putrescine, etc. Alternatively, putrescine can be formed by agmatine deimination, which itself is the decarboxylation product of arginine. These two pathways may participate in the generation of metabolic energy and/or resistance against acid stress. The decarboxylation pathways are secondary metabolic-energy-generating pathways that generate proton motive force, whereas the deiminase pathways produce ATP at substrate level and ammonia (28). The genes encoding the various decarboxylation pathways responsible for BA formation have been extensively studied in different bacterial groups and especially in lactic acid bacteria (10, 14, 15, 26, 29, 30, 32, 33). Little work has been done on the corresponding genes in CNS. Recently, histidine decarboxylase genes have been characterized in S. capitis (16) and S. epidermidis (K. Yokoi and K. Kodaira, unpublished data; GenBank accession no. AB378754), while a fragment of a putative tyrosine decarboxylase gene was described in an S. epidermidis strain (46). Genome sequencing has demonstrated that putrescine production by ornithine decarboxylation is a trait that is abundantly found in the Gram-negative Gammaproteobacteria but rarely in Gram-positive bacteria. The pathway in Escherichia coli is an example of a well-characterized pathway at both the genetic and physiological levels (24).

In this study, the origin of the ability of S. epidermidis 2015B to form putrescine and the genetic basis of strain-to-strain variation of this trait in the S. epidermidis species were investigated.

Coagulase-negative staphylococci (CNS) strains that produced biogenic amines in formed with fermented meat products (6, 33, 34, 35, 43). Othering biogenic amine production by staphylococci have been per-
**MATERIALS AND METHODS**

Bacterial strains and culture conditions. *S. epidermidis* strain 2015B was originally isolated from a mahimahi fish fillet (1) and kindly provided by D. Green from the Center of Marine Sciences and Technology, Food Science Department, of the North Carolina State University. Six *S. epidermidis* strains related to dry sausage samples (VIII10B3, VII20B2, OB2, VIII 10B1, SV1, and S2) were obtained from the collection of the Association pour le Développement de l’Institut de la Viande (ADI) ( Clermont-Ferrand, France), while eight milk (S42) and cheese (S115, S118, and S119 from Camembert cheeses and S147, S146, S150, and S152 from Pont-l’évêque cheeses) isolates came from the ADIA Normandie collection (Villers-Bocage, France). Strains were grown in tryptic soy broth yeast extract (TSBYE) in aerobic conditions with agitation or tryptone soya yeast extract agar (TSBYA) (AES, France) and incubated at 30°C. *Lactobacillus* strain 30a ATCC 35222 (obtained from the American Type Culture Collection), a histidine decarboxylase (HDC- and ornithine decarboxylase (ODC)-positive bacterium, and *Lactobacillus brevis* IOEB 9809 (obtained from the Faculté d’Oncoologie de Bordeaux, France), a tyrosine decarboxylase (TyrDC- and agmatine deiminase (AgDI)-positive bacterium, were grown in de Man, Rogosa, and Sharpe medium (MRS) (AES, France) at 37°C and 30°C, respectively.

**Preparation of template DNA.** Bacterial cultures were first grown to an optical density at 600 nm (OD600) of 0.6. Total DNA was then extracted from bacterial cultures using 1.5 ml of culture with the Nucleoprep tissue kit (Macherey-Nagel) according to manufacturer’s instructions (50 to 100 ng of DNA was used in all PCR and RIA experiments). DNA was extracted using the Plasmid Midi-Kit (Qiagen) according to a user-developed protocol (http://www1.qiagen.com/literature/protocols/pdf/pki10.pdf). Plasmid restrictions were performed using the Avai and Xhol restriction enzymes (Fermentas) according to the manufacturer’s instructions.

**PCR amplification.** The detection of four BA-associated genes (hdc, odc, and the AgDI and TyrDC genes) was performed using a multiplex PCR method. This method corresponded to a modification of the multiplex PCR method previously described by Coton and Coton (12) targeting the TyrDC and hdc genes with the specific primers TD2/TD5 and HDC3/HDC4 as well as a PCR internal control (16S rRNA gene). Two new primer sets were added for detection on the one hand of odc genes, ODC1 (5’ NCAYARCAACAAAGYNGG 3’) and ODC2 (5’ GRTANGNTNNGCACCTCT 3’), and, on the other hand, of AgDI genes, AgD1 (5’ CAYGTNGAYGHAASAGG 3’) and AgD2 (5’ GTTTONTRATR CAGTGTAAT 3’). Primer concentrations were 0.8 µM for ODC1, ODC2, AgD1, and AgD2, 0.2 µM for TD2 and TD5, 0.12 µM for HDC3 and HDC4, and 0.05 µM for BSF8 and BSR1541. All multiplex experiments were carried out in the presence of 200 µM deoxyadenosine triphosphate (dTTP) (Invitrogen), 10 µg/ml bovine serum albumin (BSA) (Amersham), and 1 U HotMaster Tag polymerase (5 PRIME GmbH) in a final volume of 50 µl. The amplification program was as follows: 95°C for 5 min, 35 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min.

**Acquisition of unknown sequences adjacent to the odc gene was performed using a restriction site-PCR (RS-PCR) method.** The method corresponded to the multiplex RS-PCR method described by Weber et al. (47), with a single modification: PCR primers corresponding to the known sequence used for the second PCR were chosen, getting a 900-bp partial gene fragment, were designed based on the odc sequences of *Lactobacillus acidophilus* NCFM CP000033, *Lactobacillus gasseri* ATCC 33232 CP000413, *Lactobacillus johnsonii* NCC 533, *Lactobacillus 30a* LSU11816, and *Oenococcus oeni* ATJ461615. These strains were located at positions 1116 to 1133 and 2023 to 2038 on the *O. oeni* odc gene sequence. For the agmatine deiminase pathway, a 600-bp partial AgDI gene fragment was designed based on the odc sequences of *Lactobacillus acidophilus* NCFM CP000033, *Lactobacillus gasseri* ATCC 33232 CP000413. These primers were located at positions 478 to 494 and 1060 to 1078 on the *P. pentosaceus* AgDI gene sequence. Only in the *S. epidermidis* 2015B strain, amplification of a specific band with a size comparable to the one of *Lactobacillus 30a* (~900 bp), used as a positive control (Fig. 1), was observed.

**RESULTS**

Genotypic and phenotypic characterization of putrescine production by *Staphylococcus epidermidis* strains. The ability to produce biogenic amines, including histamine, tyramine, and putrescine (from either ornithine or agmatine), was evaluated in *S. epidermidis*. Total DNA isolated from 15 strains of food-related *S. epidermidis* was assayed for the presence of genes corresponding to histidine decarboxylase (hdc), tyrosine decarboxylase (the TyrDC gene), ornithine decarboxylase (ode), and agmatine deiminase (the AgDI gene) using a multiplex PCR method. This method corresponds to a modification of a previously described multiplex PCR method targeting TyrDC and hdc genes (12) by the addition of two new primers targeting odc and AgDI genes. Consensual primers ODC1/ODC2, targeting a 900-bp partial odc gene fragment, were designed based on the odc sequences of *Lactobacillus acidophilus* NCFM CP000033, *Lactobacillus gasseri* ATCC 33232 CP000413, *Lactobacillus johnsonii* NCC 533, *Lactobacillus 30a* LSU11816, and *Oenococcus oeni* ATJ461615. These strains were located at positions 1116 to 1133 and 2023 to 2038 on the *O. oeni* odc gene sequence. For the agmatine deiminase pathway, a 600-bp partial AgDI gene fragment was targeted with the consensual primers AgDI1/AgDI2 based on known sequences of *Pediococcus pentosaceus* ATCC 25745 CP000422, *L. brevis* IOEB 9809 AF446085, *Listeria monocytogenes* 4b F2365 AE017262, and *Lactobacillus sakei* 23K CR936503. These primers were located at positions 478 to 494 and 1060 to 1078 on the *P. pentosaceus* AgDI gene sequence. Only in the *S. epidermidis* 2015B strain, amplification of a specific band with a size comparable to the one of *Lactobacillus 30a* (~900 bp), used as a positive control (Fig. 1), was observed.
The ability of the 15 strains to form biogenic amines in vitro was evaluated on the improved detection medium (5) supplemented with six precursor amino acids (histidine, lysine, ornithine, phenylalanine, tryptophan, and tyrosine) and measured by HPLC. The S. epidermidis 2015B culture supernatant was the only one to contain significant amounts of biogenic amines, namely, putrescine (1,460.85 μg/ml) and, to a lesser extent, cadaverine (146.92 μg/ml). Remarkably, while S. epidermidis 2015B (isolated from a mahimahi fish basket) was described as a histamine-forming strain (1), only a very minor amount of this amine (4.03 μg/ml) was produced. Two strains, namely, OB2 and S42, produced minor amounts of tryptamine (6.01 and 14.00 μg/ml, respectively) and phenylethylamine (26.10 and 26.34 μg/ml, respectively).

To localize the putrescine production pathway, plasmid DNA was extracted from the S. epidermidis 2015B strain followed by digestion using the restriction enzyme AvaI or XhoI. After electrophoresis, a PCR using the odc primer set was performed on each of the obtained bands by directly targeting each band in the gel as DNA matrix. For both the AvaI and XhoI restrictions, the top band allowed for the amplification of the odc fragment (Fig. 2). The results strongly suggest that a catabolic ornithine decarboxylation pathway encoded on a plasmid is responsible for putrescine formation by S. epidermidis 2015B.

Characterization of the odc region. The obtained PCR fragment identifying the odc gene in S. epidermidis 2015B was sequenced and consisted of 832 bp. In order to determine the complete sequence of the S. epidermidis 2015B odc gene as well as of its flanking regions, a multiplex restriction site-PCR (RS-PCR) method, based on the one proposed by Weber et al. (47), was used. The method allows for rapid acquisition of unknown DNA sequences adjacent to a known segment in both the 5’ and 3’ directions. The repetitive use of this method by the creation of new sets of primers based on each newly determined sequence allowed us to obtain a 6,292-bp-long nucleotide sequence (GenBank accession no. GU799625) from the original 832-bp odc partial sequence. Sequence analysis of the fragment revealed the presence of three complete open reading frames (ORFs) flanked by two partial ORFs (Table 1).

The odc gene consisted of 2,175 bp encoding a 724-amino-acid protein exhibiting an ornithine decarboxylase family conserved domain. Sequence identities on the order of 60 to 65% were observed with the known ornithine decarboxylases of Gram-positive bacteria, i.e., Oenococcus oeni (AJ746165) and Lactobacillus 30a (LSU11816), as well as ODCs of Gram-negative bacteria, including Haemophilus influenzae (L42023) and Dichelobacter nodosus (CP000513). Comparison of the translated sequence to the ODC sequence of Lactobacillus 30a, for which the crystallographic structure has been determined (37), indicated that the residues involved in enzymatic activity were conserved (31, 38), including the pyridoxal-5-phosphate binding domain and the residue involved in the association of dimers into dodecamers. Although the S. epidermidis 2015B ODC showed the highest percent identity with the O. oeni ODC (GenBank accession no. CAG34069), it lacks the N-terminal extension of the latter (31).

Immediately downstream of the odc gene at an intergenic distance of only 22 bp, and therefore certainly cotranscribed, an open reading frame encoding a 442-amino-acid-long protein was found. The putative protein shares 59% sequence identity with the functionally characterized putrescine-ornithine antiporter (PotE) of E. coli. Homologues of PotE are found downstream of the ODC-encoding genes of O. oeni (CAM07323) and various Gram-negative bacteria as well (Fig. 3). It is likely that the two genes form a single transcriptional unit encoding the ornithine decarboxylation pathway. The same operon structure is not found around the odc genes found in a group of lactobacilli. Rather, an amino acid trans-
Amino acids (aa), theoretical molecular mass, and isoelectrical point, the last two as estimated by the "compute pI/Mw tool" (http://www.expasy.ch/tools/pi_tool.html).


Identical amino acid percentage between the predicted sequence and the closest sequence in GenBank using BlastP.

Functional expression of potE in Lactococcus lactis. The potE gene of S. epidermidis was cloned in the NICE expression vector pNZ8048 (plasmid named pNZpotE-Se) for nisin-inducible expression in L. lactis NZ9000 (17). At a concentration of 17.5 μM [14C]-ornithine, the host cells containing the empty vector pNZ8048 took up ornithine at an initial rate of 2.7 nmol ⋅ min⁻¹ ⋅ mg⁻¹, demonstrating the presence of an endogenous ornithine transport system in L. lactis NZ9000 (Fig. 4A). L. lactis cells expressing the S. epidermidis transporter gene showed a higher initial rate of ornithine uptake of 5.0 nmol ⋅ min⁻¹ ⋅ mg⁻¹, demonstrating that potE encoded an ornithine transport protein. Even more convincing was the uptake of putrescine at an initial rate of 7.8 nmol/min ⋅ mg at a concentration of 4.5 μM by the recombinant strain, while the host strain did not take up significant amounts of [14C]putrescine (Fig. 4B, circles).

The function of PotE in the ornithine decarboxylation pathway is the combined uptake of ornithine and excretion of putrescine. To demonstrate that the transporter catalyzes ornithine/putrescine exchange, cells were allowed to take up [14C]-labeled putrescine until a plateau was reached, after which an approximately 200-fold excess of unlabeled ornithine was added. The result was a rapid release of [14C]putrescine from

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the cells, showing the exchange mode of transport (Fig. 4B, triangles).

**DISCUSSION**

Concerning biogenic amine production by staphylococci, several studies have been performed in various foodstuffs. In fermented meat products, the results obtained are contrasted; while the ability of strains of *S. carnosus* or *S. xylosus* to form BA was reported in dry sausages (34, 35, 43), other studies indicated that no or few BA producers were found among CNS strains in the same type of products (6, 33). In fish-related samples, BA-producing CNS strains were also observed and belonged mainly to the *S. epidermidis* and *S. capitis* species in salted anchovies (22) and *S. hominis* in temperature-abused tuna (18). BA production by CNS in dairy products does not seem to constitute a safety problem so far (25), although Straub et al. (44) reported that an *S. epidermidis* strain isolated from a cheese surface was able to produce BA.

In this study, the genotypic and phenotypic characterization of food-related *S. epidermidis* isolates for biogenic amine production indicated that only 1 out of 15 strains was able to produce significant amounts of biogenic amines (putrescine and, to a lesser extent, cadaverine), thus indicating the existence of a strain-to-strain variation in the ability to form BA in this species.

Some authors have already shown the existence of a strain-dependent ability to form BA in various bacterial species. For instance, the ability to form histamine was shown to be strain dependent in *O. oeni* (14) and *Lactobacillus hilgardii* (30). In the latter case, the amine-producing pathway was harbored by an unstable plasmid (30), whereas for tyramine production, the presence of a putative genomic island harboring the complete TyrDC operon was shown to be associated with the ability to form tyramine in *L. brevis* (13). Concerning putrescine production, Marcobal et al. (32) recently demonstrated that the ability to form putrescine in *O. oeni* was a strain-dependent trait and that the involved genes, the ornithine decarboxylase, *odc*, and the putrescine-ornithine antiporter, *potE*, were situated at the chromosomal level in a region that was certainly acquired through horizontal gene transfer (HGT).

For *S. epidermidis* 2015B, the presence of a sequence corresponding to a mobilization protein (MobC) was observed upstream of the *odc* gene. In *S. aureus*, MobC has been shown to correspond to a nicking accessory protein belonging to the relaxosome complex, also constituted by the MobA (DNA relaxase) and MobB (mobilization accessory protein) proteins, that allows for the preparation of plasmid transfer between bacterial cells by conjugation. MobC proteins have been shown to possess binding specificity for the origin of transfer (oriT) and therefore contribute to the site- and strand-specific cleavage at a unique nick site (nic) within the oriT performed by the relaxosome (8). The obtained mobC sequence, also found on various *S. epidermidis* plasmids, suggested that the putative putrescine production pathway could be carried by a plasmid.

Comparison to an international data bank of the 628-bp intergenic sequence situated between the putative cation efflux protein and IS431mec-like transposase gene, the 306 leftmost base pairs showed a high degree of identity (91%) with *S. epidermidis* plasmidic fragments (i.e., GenBank accession no. GQ900505). For the intergenic sequence situated between the putative cation efflux protein and IS431mec-like transposase gene, the 306 leftmost base pairs showed a high degree of identity (>91%) with *S. epidermidis* plasmidic fragments (i.e., GenBank accession no. GG900505 and AF045240). Therefore, the obtained sequences as well as experiments performed on the plasmid extraction confirmed that the putrescine production pathway, consisting at least of the *odc* and *potE* genes, is carried by a plasmid in *S. epidermidis* strain 2015B and was thus acquired through HGT. This localization explains the fact that the putrescine production trait is observed to be strain dependent in the *S. epidermidis* species.

Remarkably, as already observed for *O. oeni* (32), the ge-

![FIG. 3. Phylogenetic tree comparing the protein sequences of amino acid transporters associated with ornithine decarboxylases. The tree was constructed using the Pearson correlation; unweighted-pair group method using average linkages (UPGMA) and bootstrap values are indicated at branch points (percentages of 1,000 replications).](image-url)
The role of biogenic formation by amino acid decarboxylation is believed to protect against acid stress and/or to generate metabolic energy (48). The pathways that consist of a decarboxylase and an amino acid/biogenic amine exchanger generate proton motive force through proton consumption in the cytoplasmic decarboxylation reaction and membrane potential generation through electrogenic exchange of the precursor amino acid and the corresponding biogenic amine (36). In this context, Azcarate-Peril et al. (3) identified the odc gene to be involved in acid tolerance in L. acidophilus NCFM by challenging mutants with a variety of acidic conditions, and Pereira et al. (40) demonstrated the involvement of ODC in proton motive force generation. Bover-Cid et al. (7) indicated that ornithine decarboxylase in Lactobacillus curvatus CTC273 would not seem to be a mechanism to neutralize the acid environment but may rather play a role in supplying metabolic energy.

Finally, the presence, downstream of the putrescine production pathway, of a cation efflux protein-encoding gene involved in detoxification action towards toxic metallic ions raises the question of the existence of a resistance island (combining the putrescine production pathway for acid stress resistance and the cation efflux protein for tolerance to toxic metallic ions), harbored by a plasmid, in S. epidermidis 2015B. The effect of the presence of these genes on the adaptability and growth of S. epidermidis 2015B in various environmental conditions should be further investigated.

ACKNOWLEDGMENTS

We are grateful to C. Desmarais, N. Buron, and F. Revarddeau for their technical assistance, to D. Green of the Center of Marine Sciences and Technology, Food Science Department, of the North Carolina State University for providing strain 2015B, and to S. Christians of ADIV for providing the dry-sausage-associated strains.

This work was funded by the French National Research Agency (ANR) (project ANR-05-PNRA-005) and by the European Community’s Seventh Framework Program, grant agreement no. 211441-BI-AMFOOD.

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