Tyrosine decarboxylase activity of Enterococcus mundtii: new insights into phenotypic and genetic aspects

Veronica Gatto, Giulia Tabanelli, Chiara Montanari, Valentina Prodomi, Eleonora Bargossi, Sandra Torriani and Fausto Gardini

1 Department of Biotechnology, University of Verona, Verona, Italy.
2 Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy.
3 Interdepartmental Center for Industrial Agri-food Research, University of Bologna, Cesena, Italy.

Summary

Few information is available about the tyraminogenic potential of the species Enterococcus mundtii. In this study, two plant-derived strains of E. mundtii were selected and investigated to better understand the phenotypic behaviour and the genetic mechanisms involved in tyramine accumulation. Both the strains accumulated tyramine from the beginning of exponential phase of growth, independently on the addition of tyrosine to the medium. The strains accumulated also 2-phenylethylamine, although with lower efficiency and in greater extent when tyrosine was not added. Accordingly, the tyrosine decarboxylase (tyrDC) gene expression level increased during the exponential phase with tyrosine added, while it remained constant and high without precursor. The genetic organization as well as sequence identity levels of tyrDC and tyrosine permease (tyrP) genes indicated a correlation with those of phylogenetically closer enterococcal species, such as E. faecium, E. hirae and E. durans; however, the gene Na+/H+ antiporter (nhaC) that usually follow tyrP is missing. In addition, BLAST analysis revealed the presence of additional genes encoding for decarboxylase and permease in the genome of several E. mundtii strains. It is speculated the occurrence of a duplication event and the acquisition of different specificity for these enzymes that deserves further investigations.

Introduction

Tyramine is a biogenic amine (BA) deriving from tyrosine decarboxylation and can have severe acute effects if ingested in excessive amounts with food, consisting in peripheral vasoconstriction, increased cardiac output, accelerated respiration, elevated blood glucose and release of norepinephrine, symptoms known also as ‘cheese reaction’ (Shalaby, 1994; McCabe-Sellers et al., 2006; Marcobal et al., 2012). Tyrosine decarboxylase, the enzyme responsible for tyramine production, can use as substrate also phenylalanine, producing 2-phenylethylamine, whose adverse effects are similar to tyramine (Marcobal et al., 2006).

In general, the amino acid decarboxylation leading to BA formation provides metabolic energy and/or resistance against acid stress (Molenaar et al., 1993; Fernández and Zúñiga, 2006; Pereira et al., 2009). The microorganisms responsible for tyramine accumulation in foods belong mainly to the group of lactic acid bacteria (LAB) (Marcobal et al., 2012). Among LAB, species belonging to the genus Enterococcus are recognized as the most frequent and intensive tyramine producers (Leuschner et al., 1999; Suzzi and Gardini, 2003; Ladero et al., 2012).

Due to their salt and pH tolerance, and to their ability to grow over a wide temperature range, enterococci are isolated from different habitats and are often contaminants in food of animal origin, such as cheese and sausages (Giraffa, 2003; Franz et al., 2011). In spite of their homolactic metabolism, their potential role in cheese ripening and their ability to produce bacteriocins (Beshkova and Frengova, 2012; Fontana et al., 2015), enterococci have a controversial status and they are often considered at the crossroad of food safety (Franz et al., 1999). In fact, this group is considered as indicator of the hygienic quality of raw material and food, as well as marker of faecal contamination (Leclerc et al., 1996). In addition, virulence factors can be present (Foulquié Moreno et al., 2006; Hollenbeck and Rice, 2012) and they can act as opportunistic human pathogens frequently associated with nosocomial infections due to their antibiotic resistance with a high capacity to disseminate this.
resistance to other microorganisms (Giraffa, 2002; Klein, 2003; Rossi et al., 2014). Furthermore, they are strong tyramine producers and this ability has been deeply exploited in Enterococcus faecalis (in which tyramine production is considered a species trait), Enterococcus faecium and Enterococcus durans (Linares et al., 2009; Ladero et al., 2012; Bargossi et al., 2015a,b). For this reason, the presence of enterococci has been put in relation with the presence of tyramine in several fermented foods, such as fermented sausages (Gardini et al., 2008), cheeses (Linares et al., 2011) and wine (Pérez-Martin et al., 2014). The enterococcal species most frequently isolated from fermented foods are E. faecalis and E. faecium, and also E. durans, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus hirae can be found in food matrices (Franz et al., 2003; Giraffa, 2003; Foulquie Moreno et al., 2006; Corsetti et al., 2007; Komprda et al., 2008). Recently, also E. mundtii has been isolated from the food chain; it is a non-motile, yellow-pigmented enterococcus infrequently associated to human infection (Collins et al., 1986; Higashide et al., 2005). Strains of E. mundtii have been isolated from soy and cereals (Todorov et al., 2005; Corsetti et al., 2007), water (Moore et al., 2008; Graves and Weaver, 2010; Furtula et al., 2013), soil (Collins et al., 1986; Bigwood et al., 2012) and forage grass or silage, in which this species is often the predominant among enterococci (Muller et al., 2001; Ni et al., 2015).

It has also been isolated from animals (Collins et al., 1986; Espeche et al., 2014) and from food (Vera Pingitore et al., 2012; Schobitz et al., 2014). This species has been deeply studied in relation to the bacteriocin produced, among which mundticine (De Kwaadsteniet et al., 2005; Todorov et al., 2005; Corsetti et al., 2007; Feng et al., 2009; Vera Pingitore et al., 2012; Espeche et al., 2014).

Recently, the genome of E. mundtii QU 25, an efficient L-lactic acid-producing bacterium isolated from ovine faeces, has been completely sequenced (Shiwa et al., 2014), and comparative analysis of the genetic content of this species with respect to other representative enterococcal species of diverse origins was conducted (Repizo et al., 2014). Despite to those recent acquisitions, scarce information is available about E. mundtii tyraminogenic potential. Trivedi et al. (2009) carried out a study testing the ability to decarboxylate tyrosine in several enterococci isolated from different foodstuffs. Regarding E. mundtii, four of five strains isolated from meat products and six of 12 isolated from vegetables and fruits possessed this ability. Also Kalihotka et al. (2012) found an E. mundtii strain able to produce tyramine and agmatine. This latter amine derives from the decarboxylation of arginine and can be transformed in putrescine by a specific deiminase (Linares et al., 2015).

In this research, the tyramine and 2-phenylethylamine accumulation by two E. mundtii strains isolated from grass silage was studied during their growth in a rich medium. In addition, information on the genetic basis of the tyraminogenic potential of E. mundtii were obtained analysing the expression of the tyrosine decarboxylase (tyrDC) gene, the sequence of tyrDC and tyrosine permease (tyrP) genes, and the genetic organization of the TDC operon region.

**Results and discussion**

**Tyramine-positive enterococci**

In the first part of the research, 35 isolates of coccace LAB, originating from different agricultural foodstuffs (Fig. 1) and positive for the production of tyramine according to the method of Bover-Cid and Holzapfel medium (Bover-Cid and Holzapfel, 1999) were considered. These isolates were presumptively identified as enterococci based on their physiological and morphological characteristics (von Wright and Axelsson, 2012). They were cocci, Gram-positive, catalase-negative, non-spore-forming and occurring both as single cells and in chains. They were able to grow at 10°C and 45°C, at pH 4.4 and 9.6, and in the presence of 6.5% of NaCl.

To confirm the decarboxylase activity revealed by the Bover-Cid and Holzapfel medium, the occurrence of the gene tyrDC, coding for tyrosine decarboxylase (TDC), was examined. A tyrDC gene fragment was amplified according to Torriani et al. (2008). For all the 35 isolates, the 336 bp amplicon was obtained, confirming their tyraminogenic potential.

Successively, RAPD-PCR fingerprinting technique with the primer 1254 (Table 1) was applied to investigate the genetic diversity of the strains. This molecular typing method has proved to be reliable, discriminative and suitable for the study of a large number of strains in short time (Vancanneyt et al., 2002). The primer 1254 generated reproducible RAPD-PCR fingerprints thanks to an accurate standardization of all the PCR and electrophoresis conditions. The reproducibility of PCR assays and running conditions, estimated by analysis of duplicate DNA extracts of several strains, was higher than 90%. Cluster analysis of the RAPD-PCR fingerprints grouped the 35 isolates in three clusters (Fig. 1). Seven strains, all originated from ryegrass silage except one (C46), were grouped in the cluster I, four strains from ryegrass and maize grain silages belonged to the cluster II and, finally, 24 strains from different foodstuffs were clustered in the group III.

For each cluster, some representative isolates were chosen to proceed with their identification at the species level by the pheS gene analysis. Indeed, this gene is considered a reliable genomic marker for differentiating the species within the genus Enterococcus, and it was
demonstrated to be much more discriminatory than 16S rRNA (Naser et al., 2005). The \textit{pheS} gene has a high degree of homogeneity among strains of the same enterooccal species (at least 97\% sequence similarity), whereas distinct species reveal at maximum 86\% gene sequence similarity. The \textit{pheS} partial gene sequence data obtained indicated that the strains C46, C53 and C77, grouped in the cluster I, can be assigned to the species \textit{E. mundtii} (99–100\% identity), the strain E599 (cluster II) to \textit{E. faecalis} (100\% identity), while the strains E175, G52 and C5 (cluster III) to \textit{E. faecium} (100\% identity). After that, the analysis of the \textit{pheS} gene was extended to all the isolates of cluster I, thus confirming their belonging to the \textit{E. mundtii} species.

These results confirmed the tyrosine decarboxylase potential of \textit{E. faecalis} and \textit{E. faecium}, the stronger tyramine producers (Aymerich et al., 2006; Bonetta et al., 2008; Gardini et al., 2008; Ladero et al., 2012; Marcobal et al., 2012). On the other hand, tyramine production is considered a species characteristic of \textit{E. faecalis} (Ladero et al., 2012). In addition, the tyraminogenic potential of \textit{E. durans} has been deeply studied (Fernández et al., 2007; Linares et al., 2009). Regarding \textit{E. mundtii}, scarce are the studies on their capability to accumulate tyramine and the genetic aspects involved in its accumulations. Kalhotka et al. (2012) investigated the decarboxylase activity of enterococci isolated from goat milk and found that all of the tested strains, identified as \textit{E. mundtii}, \textit{E. faecium} and \textit{E. durans}, showed significant tyrosine and arginine decarboxylase activity, in relation to temperature and duration of cultivation. In addition, Trivedi et al. (2009) studied the ability to decarboxylate tyramine in many enterococcal strains isolated from different foodstuffs and found that more than 90\% of isolates

![UPGMA dendrogram derived from RAPD-PCR-fingerprinting patterns of all the 35 isolates using the primer 1254. Code and source of the isolates are indicated on the right-hand side of the figure. The vertical dotted line indicates the 60\% similarity level that delineates the species \textit{E. mundtii} (cluster I), \textit{E. faecalis} (cluster II) and \textit{E. faecium} (cluster III). Isolates marked with \* were identified by phenylalanyl-tRNA syn-...](image)
showed the presence of the gene tyrDC. In particular, these authors found that 10 of 17 *E. mundtii* strains were tyramine producers. These preliminary studies indicated the occurrence of tyramine-producing *E. mundtii* strains, but did not highlight the tyraminogenic potential of this species. Moreover, the molecular aspects involved in the tyramine biosynthesis have not yet studied in depth. For this reason, two of the *E. mundtii* strains considered here were chosen as targets for investigating their tyramine accumulation capability and tyrosine metabolism. In particular, the two strains C53 and C46 were selected on the basis of their different origin and genetic diversity. Indeed, these strains have limited genetic similarity, belonging to different RAPD-PCR subclusters, as shown in Fig. 1; in addition, C53 was the sole *E. mundtii* strain of the collection that originated from lucerne silage.

**Growth parameters and tyramine production of Enterococcus mundtii strains**

The growth of the strains *E. mundtii* C46 and C53 was monitored by measuring the OD$_{600}$ increase in BHI medium added or not with tyrosine. The OD$_{600}$ changes were modelled with the Gompertz equation (Zwietering et al., 1990) and the estimates of the parameters are reported in Table 2. All the parameters were characterized by a high significance ($P \leq 0.05$). Both the strains reached the maximum value of OD$_{600}$ ($A$), ranging between 1.11 and 1.27, after 6–8 h incubation at 37°C. The curves presented a very short lag phase ($\lambda$), followed by a sharp increase of OD$_{600}$. As far as $A$ and $\lambda$, no marked differences were found among the two strains, while *E. mundtii* C53 presented a lower maximum OD$_{600}$ increase rate in exponential phase ($\mu_{\text{max}}$). Moreover, the addition of tyrosine generally determined lower values of $A$, higher values of $\mu_{\text{max}}$ and a shorter lag phase. Table 2 reports also the cell counts detected at beginning of the stationary phase. The models obtained are graphically represented in Fig. 2, which reports the growth curves in the first 24 h of incubation. As a reference, the same figure shows also the growth curves obtained under the same conditions by Bargossi et al. (2015b) for *E. faecalis* EF37, a strong tyramine producer (Gardini et al., 2008), which exhibited analogous behaviours.

The production of tyramine by *E. mundtii* C46 and C53 during their growth in BHI, added or not with the precursor, is shown in Table 3, which reports also the accumulation of 2-phenylethylamine. Also in this case, the data already available for *E. faecalis* EF37 (Bargossi et al., 2015b) are reported. It is well known that enterococci can decarboxylate phenylalanine producing 2-phenylethylamine through the activity of the same decarboxylase. The characteristics of this BA are very similar to tyramine, but it is produced with a lower efficiency (Marcobal et al., 2006).

In all the tested conditions, the two *E. mundtii* strains were able to accumulate tyramine independently on the addition of tyrosine. In fact, the decarboxylase activity was detected also in the medium not supplemented with tyrosine, because BHI contains amino acid sources (proteins and peptides) among which precursors for TDC. This observation was previously reported by Bargossi et al. (2015b) for *E. faecalis* and *E. faecium* grown in the media BHI and Bover-Cid and Holzapfel.

The data showed that the two *E. mundtii* strains began to produce tyramine after 2 h from the inoculum, both in the presence and in the absence of the precursor, and

| PCR type   | Target     | Primer code | Sequence (5'–3') | Amplicon (pb) | Reference                  |
|------------|------------|-------------|------------------|---------------|---------------------------|
| RAPD-PCR   | Arbitrary DNA sequences | 1254 | CCG CAG CCA A | Variable       | Akopyanz et al. (1992)    |
| RT-qPCR    | tyrDC      | TYR3f       | CGT ACA CAT TCA GTT GCA TGG CAT | 171           | Torriani et al. (2008)    |
|            |            | TYR4r       | ATG TCC TAC TTC TTC TTC CAT TTG |               |                           |
| Conventional | tyrDC     | DEC5       | CGT TGT TGG TGT TGG CAC NAC | 350           |                           |
|            | pheS       | pheS-21-F   | CAY CON GCH CGY GAY ATG C | 455           | Naser et al. (2005)       |
|            |            | pheS-22-R   | CCW ARV CCR AAR GCA AAR CC |               |                           |
|            | tyrS/tyrDC | TyrS-F1     | GGA GCT ATA AGT ATT AAC GGT GA | 940           | Bargossi et al. (2015a)   |
|            |            | Tdc-R1      | GAT TT(A/G) ATG TT(A/G) CG(G/C) GCA TAC CA |               |                           |
|            | tyrDC      | Tdc-F2      | CAA ATG GAA GAA GAA GT(A/T) GGA | 1340          |                           |
|            |            | Tdc-R2      | CC(A/G/T) GCA CG(G/T) T(C/T)C CAT TCT TC |               |                           |
|            | tyrDC| Tdc-R3      | CCT AAA GTA GAA GC(A/G) ACC AT | 788           |                           |
|            | tyrP       | TyP-F3      | CAT C(T/G) TAT GGC AA(C/T) AGC CCA | 940           |                           |
|            |            | TyP-F4      | TGG GTG CAA ATG TTC CCA GG |               |                           |
|            |            | TyP-R3      | ACC (A/G)AT TCG (A/G)TA AGG ACG |               |                           |
|            |            | TyP-R4      | (A/T)CT GCT TGG GT(A/T) ACT GGA CC | na            |                           |
|            |            | NhaC-R5     | CAT (C/T)GC AT(C/T) GGT(T/C) GAA TAC CAG |               |                           |
they continued to gradually accumulate tyramine during their stationary phase. In all the conditions, the maximum tyramine concentration was reached after 48 h for the strain C46 and after 72 h for the strain C53. However, the final amount of tyramine was similar for both the strains. In fact, it did not exceed 135 mg l\(^{-1}\) in BHI medium, while in the presence of tyrosine, the final amount of tyramine was about 767 and 797 mg l\(^{-1}\) for the strains C53 and C46 respectively. As reported in Table 3, Bargossi et al. (2015b) found that *E. faecalis* EF37 under the same conditions after 8 h reached the maximum tyramine concentration in the presence of tyramine added. The *E. mundtii* strains showed a slower tyramine production kinetics, but the final amount was significantly higher than *E. faecalis* EF37 (approximately 500 mg l\(^{-1}\)). In the absence of tyrosine added, the strain *E. mundtii* C46 was characterized by a faster tyramine accumulation in BHI. The major differences between *E. faecalis* EF37 and the *E. mundtii* strains were in the ability to accumulate 2-phenylethylamine, which was dramatically higher in *E. faecalis*. These amounts were higher than those reported by Liu et al. (2013) who, testing the tyraminogenic potential of *E. faecalis* strains from water-boiled salted duck, found concentrations of tyramine lower than 330 mg l\(^{-1}\) in MRS broth added with 0.1% tyrosine.

The two *E. mundtii* strains were also able to decarboxylate phenylalanine leading to the production of 2-phenylethylamine (Table 3). This BA was accumulated only after 24 h of growth for the strain C53, while C46
Table 3. OD₆₀₀ and tyramine (TYR) and 2-phenylethylamine (2-PHE) production by *E. mundtii* C53 and C46 during their growth in BHI, added or not with 1% tyrosine. It is also reported the production of TYR and 2-PHE by *E. faecalis* EF37 strain (adapted from Bargossi et al., 2015b). The standard deviations are reported within parentheses.

| Time (h) | BHI | BHI + 0.1% tyrosine | BHI | BHI + 0.1% tyrosine | BHI | BHI + 0.1% tyrosine |
|---------|-----|---------------------|-----|---------------------|-----|---------------------|
|         | OD₆₀₀.b |TYR (mg l⁻¹) | 2-PHE (mg l⁻¹) | OD₆₀₀ | TYR (mg l⁻¹) | 2-PHE (mg l⁻¹) | OD₆₀₀ | TYR (mg l⁻¹) | 2-PHE (mg l⁻¹) | OD₆₀₀ | TYR (mg l⁻¹) | 2-PHE (mg l⁻¹) |
| 2       | 0.000 | 8.35 (± 0.41)     | 0.000 | 20.31 (± 0.32)     | 0.004 | 7.14 (± 0.19)     | 0.167 | 15.66 (± 0.65)     | 0.059 | n.d.     | n.d.     |
| 3       | 0.367 | 21.30 (± 1.12)    | 0.575 | 42.18 (± 1.05)     | 0.279 | 21.56 (± 0.72)    | 0.748 | 72.89 (± 2.04)     | 0.575 | n.d.     | n.d.     |
| 4       | 0.865 | 32.16 (± 1.84)    | 0.953 | 64.88 (± 1.54)     | 0.846 | 36.59 (± 0.08)    | 1.047 | 130.34 (± 2.56)    | 0.913 | n.d.     | n.d.     |
| 5       | 1.103 | 46.29 (± 1.70)    | 1.073 | 93.59 (± 2.33)     | 1.139 | 61.37 (± 1.81)    | 1.128 | 189.87 (± 3.63)    | 1.004 | n.d.     | n.d.     |
| 8       | 1.212 | 72.25 (± 2.31)    | 1.112 | 221.25 (± 5.48)    | 1.267 | 97.55 (± 3.68)    | 1.153 | 396.36 (± 3.80)    | 1.029 | 11.65     | 39.67     |
| 24      | 1.215 | 101.71 (± 3.44)   | 1.113 | 508.88 (± 0.48)    | 1.269 | 112.33 (± 0.60)   | 1.153 | 630.09 (± 0.50)    | 1.029 | 90.97     | 177.10    |
| 48      | 1.215 | 116.73 (± 6.78)   | 1.113 | 691.44 (± 0.83)    | 1.269 | 121.42 (± 4.32)   | 1.153 | 770.35 (± 4.24)    | 1.029 | 69.64     | 213.79    |
| 72      | 1.215 | 129.12 (± 4.09)   | 1.113 | 757.43 (± 0.87)    | 1.269 | 127.57 (± 0.96)   | 1.153 | 871.50 (± 0.95)    | 1.029 | 68.30     | 262.46    |
| 96      | 1.215 | 134.15 (± 2.11)   | 1.113 | 786.57 (± 4.09)    | 1.269 | 129.46 (± 2.22)   | 1.153 | 797.28 (± 1.24)    | 1.029 | 94.70     | 405.80    |

a. Adapted from Bargossi et al. (2015b).

b. Optical density at the different sampling time as predicted by the Gompertz model (Table 2).

c. Under the detection limit (0.5 mg l⁻¹).

d. n.d., not determined.
began to produce this compound already after 8 h in the absence of tyrosine. The 2-phenylethylamine accumulation increased during subsequent incubation and reached its maximum level after 72 h with amended tyrosine and after 96 h without this amino acid. Moreover, the production of 2-phenylethylamine was higher when tyrosine was not added to the growth medium. Indeed, in this case, concentrations of about 76 and 109 mg l⁻¹ for *E. mundtii* C53 and C46, respectively, were reached, compared with concentrations lower than 45 mg l⁻¹ in BHI when tyrosine was added to the medium. Interestingly, however, the accumulation of this BA became relevant when the tyramine concentration reached its maximum level (independently on the addition of the precursor). In any case, the amount of this BA was lower than that accumulated by *E. faecalis* FC12 and *E. faecalis* EF37 (more than 400 mg l⁻¹) grown in the same medium (Bargossi et al., 2015b). These findings could reflect the lower efficiency of the *E. mundtii* TDC for phenylalanine decarboxylation and could indicate that these amounts of tyramine can lower or inhibit further decarboxylase activities in the tested strains.

The continuous tyramine accumulation until late stationary growth phase observed in this research could represent an advantage for the microorganism against acidification during the fermentation process and growth. In fact, the decarboxylation of amino acids has been indicated as a mechanism through which LAB and human pathogenic bacteria can resist acidic conditions (Lund et al., 2014; Romano et al., 2014) and this protective effect seems to be mediated via the maintenance of intracellular pH (Perez et al., 2015). The same role in the maintenance of pH homeostasis in acidic environment has been also described in *E. durans* (Linares et al., 2009) and *E. faecium* (Marcobal et al., 2006).

**Time-course of tyrDC gene expression**

Table 4 reports the *tyrDC* gene expression data obtained for *E. mundtii* C46 and C53 by RT-qPCR during 72 h growth in BHI supplemented or not with tyrosine. The *tyrDC* gene expression data previously obtained for *E. faecalis* EF37 by Bargossi et al. (2015b) are also reported as a reference.

In general, the *tyrDC* gene expression time-course did not differ considerably between the two *E. mundtii* strains, even if the values found for the strain C53 were averagely lower. These data are in compliance with the phenotypic behaviour of the two analysed *E. mundtii* strains, as they showed similar trends in the accumulation of tyramine and phenylethylamine, and produced comparable final levels of these BAs in the different tested conditions.

In the medium without tyrosine, a high value of transcript (about 3 log copies/µg cDNA) was already
observed after 2 h (early exponential phase), probably due to the strong residual effect of the precursor present in the pre-cultivation medium. The amount of tyrDC transcript remained rather stable throughout all the period monitored. The addition of the precursor affected considerably the tyrDC expression level depending on the growth phase. Indeed, the expression of tyrDC increased rapidly, peaked (> 4 log copies/µg cDNA) at 4 h during the exponential phase of growth, when the highest number of cells for ml was reached. After 8 h, the gene expression decreased progressively until the end of the 72-h period monitored.

As notice above, the *E. mundtii* strains were able to accumulate greater amounts of BAs than that of other previously studied enterococcal strains *E. faecalis* EF37 and *E. faecium* FC12 under the same conditions (Bargossi et al., 2015b). However, the maximum tyrDC gene copies number of *E. mundtii* C46 and C53, obtained after 4 h growth in BHI with tyrosine, did not reach the value found for *E. faecalis* EF37 (6.1 log copies/µg cDNA) in the same conditions. The expression trend of the *E. mundtii* strains in BHI without tyrosine was more similar to that of *E. faecium* FC12 which presented a rather constant tyrDC transcript level during the entire incubation period. However, in BHI added with tyrosine, the expression profile differed between the *E. mundtii* strains and *E. faecium* FC12 because the tyrDC gene transcript reached the maximum level in the exponential (4 h) and in the stationary phase (24 h), respectively, when the highest cell number of 9 log CFU ml⁻¹ was detected for all these strains.

**Analysis of the TDC operon region**

The characteristics of the TDC operon region involved in tyramine production have been described in several tyraminogenic bacterial strains, including enterococci (Connil et al., 2002; Lucas et al., 2003; Coton et al., 2004; Fernández et al., 2004; Marcobal et al., 2012; Bargossi et al., 2015a). However, the molecular knowledge of this region for *E. mundtii* is extremely scarce. Therefore, an investigation was carried out to determine the DNA and amino acid sequences of the *E. mundtii* C46 tyramine production-associated genes and the genetic organization of the TDC operon region, considering also the available genome sequencing data. In particular, the region downstream the gene tyrS including the genes tyrDC and tyrP, which encode for the tyrosine decarboxylase and the tyrosine/tyramine permease, respectively, was amplified and sequenced. Indeed, the gene *Natl/H⁺* antiporter (*nhaC*), that usually follow tyrP in the TDC operon of several tyramine-producing LAB, such as *E. faecalis*, *E. faecium* and *L. brevis* (Marcobal et al., 2012; Bargossi et al., 2015a) was not recognized by PCR performed with the primers covering the intergenic region between tyrP and nhaC. Such gene organization was found also in the fully sequenced and assembled genome of *E. mundtii* QU 25 (Shiwa et al., 2014) (GCA_000504125.1) that shows a lacI family transcriptional regulator gene downstream tyrP (Fig. 3A).

BLASTN analysis of the 3677 bp nucleotide sequence of the *E. mundtii* C46 TDC operon region showed the best overall identity of 99% (3673/3677 nt) with that of *E. mundtii* QU 25. High levels of DNA sequence identity (> 80%) were also found for several strains belonging to other enterococcal species: *E. hirae* ATCC 9790 (1884/2282, 83%), *E. durans* KLDS 6.0930 and KLDS 6.0933 (1876/2285, 82%), and *E. faecium* Aus0085, NRRL B-2354, Aus0004, DO, and T110 (1877/2286, 82%). On the contrary, lower sequence identity (76%) was achieved for strains belonging to the species *E. faecalis* (e.g. ATCC 29212 and V583). Putative promoter and terminator were found upstream the start codon of the genes tyrDC (Fig. 3A), but not in the short intergenic sequence before the gene tyrP, suggesting that these two genes are probably co-transcribed, as already observed.

![Fig. 3.](image)

© 2016 The Authors. *Microbial Biotechnology* published by John Wiley & Sons Ltd and Society for Applied Microbiology, *Microbial Biotechnology*, 9, 801–813
showed for other species, such as *E. faecalis* and *L. brevis* (Marcobal et al., 2012).

Surprisingly, BLASTN analysis discovered in the genome of *E. mundtii* QU 25 (Shiwa et al., 2014), the presence of another region constituted by two genes similar to tyrDC and tyrP. These genes showed lower sequence identity values, 69% and 64%, respectively, with those present in the TDC operon. The genetic organization of the genomic segment that includes these two genes is shown in Fig. 3B. This additional portion was also recovered in the genome of other enterococcal strains, such as *E. hirae* ATCC 9790, *E. faecium* NRRL B-2354, *E. durans* KLDS6.0930 and KLDS6.0930. However, in these strains a further putative amino acid permease was annotated between the tyrosine permease and the cation transporter E1-E2 family ATPase. The presence of a gene associated to a transposase after the ATPase encoding gene in *E. mundtii* QU 25 (Shiwa et al., 2014) is of particular interest, as it could be involved in spontaneous events of gene duplication or horizontal transfer.

BLASTX analysis and comparison of the deduced amino acid sequences of *E. mundtii* C46 TDC operon region were also carried out. The translated nucleotide sequence generated two proteins in the frame +1 and +2 respectively. The first one showed the highest identity with a tyrosine decarboxylase (BAO05941.1) of *E. mundtii* QU 25 (624/624 nt, 100%) and *E. mundtii* CRL35 (616/624 nt, 99%) and decreasing identity (90% to 71%) with decarboxylases from other species of the genus Enterococcus. On the contrary, lower similarity (61% to 9%) was found with the additional PLP-dependent decarboxylase detected with BLASTN analysis. The second protein presented a putative conserved domain associated to a putative glutamate/gamma-amino butyrate antipporter (TIGR03813). This sequence showed 100% identity with the amino acid permease family protein of *E. mundtii* QU 25 and *E. mundtii* ATCC 883, and decreasing identity with the amino acid permeases of other species of the genus Enterococcus. Also in this case, lower identity (58–60%) was found with the additional amino acid permease detected with BLASTN analysis.

These sequence analysis results taken together indicated the presence in the *E. mundtii* genome of a TDC operon with a classical genetic organization (i.e. tyrS, tyrDC and tyrP) and provided evidences for a new additional copy consisting of three ORF. According to Lynch and Conery (2000), duplications of a genome segments have been thought to be a primary source of material for the origin of evolutionary novelties, including new gene functions and expression patterns. Therefore, the additional copy may acquire a novel, beneficial function and become preserved by natural selection, with the other copy retaining the original function. Recently, Bargossi et al. (2015a) described the compromised tyrosine decarboxylase activity of the strain *E. faecium* FC643 due to a codon stop in the translated tyrDC sequence. However, this strain showed a slow and reduced production of tyramine, and not 2-phenylethylamine, probably due to the presence of the additional enzyme with different substrate specificity and regulation mechanism respect to the decarboxylase encoded by the gene tyrDC of the TDC operon.

As regards *E. mundtii*, it can be supposed that all the genes in the two operon regions detected are expressed and produce functional products. As BLAST analysis revealed that the primer pairs DEC5/DEC3 and TYR3f/TYR4r used in this study were able to match conserved regions on both the putative tyrDC genes present in the *E. mundtii* QU25 genome, new target-specific primers have to be designed to detect and analyse the contribute of the additional genes to the overall tyraminogenic potential of *E. mundtii*. Therefore, the role of the additional genes and proteins in the context of BA production needs further deep investigation.

Conclusions

In this study, the capability of *E. mundtii* strains to accumulate tyrosine and 2-phenylalanine in cultural media was assessed, and more information on the genetic basis of their tyraminogenic potential were obtained for the first time. The two strains considered here produced greater amounts of tyramine than those accumulated by other strains belonging to *E. faecium* and *E. faecalis* previously studied in the same conditions (Bargossi et al., 2015b). By contrast, their ability to decarboxylate phenylalanine was less enhanced if compared with the same strains. Likewise the other enterococcal strains, the expression analysis of the gene tyrDC showed that an excess of the precursor tyrosine affected the amount of the transcript during the exponential phase of growth, and that the amino acids fraction present in the medium also modulated the level of the transcript. The genetic organization as well as sequence identity levels of the genes tyrDC and tyrP indicated that the tyramine-forming pathway in *E. mundtii* is similar to those in phylogenetically closer enterococcal species, such as *E. faecium*, *E. hirae* and *E. durans*; however, the gene Na⁺/H⁺ antipporter (nhaC) that usually follow tyrP is missing. Analysis of the available data on genome content and organization of *E. mundtii* QU 25 (Shiwa et al., 2014) and other *Enterococcus* strains revealed an unexpectedly presence of another region that includes two genes encoding for an additional PLP-dependent decarboxylase and an amino acid permease. It is tempting to speculate that a duplication event occurred and the evolution of this redundant copy induced the acquisition of different specificity.
leading to the maintenance of both the functional copies. Thus, this discovery uncovers another level of complexity in the enterococcal BAs regulatory network. Further studies have to be performed to better explain the genetic and functional characteristics of these further enzymes and their correlation with tyrosine decarboxylating potential of enterococci. Moreover, regulation of decarboxylases and permeases at protein level has to be evaluated to verify if post-translational mechanisms could affect and modulate enzymatic activities.

Experimental procedures

Characterization of the strains and screening procedure for tyramine production

In the present study, we used 35 cocci LAB isolates (Fig. 1), deposited in the bacterial culture collection of the Biotechnology Department of the Verona University. They were previously isolated from different fresh and ensiled forage crops (namely lucerne, ryegrass, maize), maize grain silage and starter cultures for silages, as shown in Fig. 1. All isolates were maintained as culture stocks in 20% (w/v) glycerol at −80°C and grown aerobically in BHI Broth (Oxoid, Basingstoke, UK) at 37°C for 24 h, unless indicated otherwise.

The isolates were tested for morphological characteristics, Gram test, catalase test, growth in the presence of 6.5% NaCl, growth at 15 and 45°C and at pH 4.4 and 9.6, as well as for their homo or heterolactic fermentation.

The tyrosine decarboxylase activity of the isolates was evaluated using the screening plate method described by Bover-Cid and Holzapfel (1999).

TyrDC gene detection

Genomic DNA of tyramine-positive isolates was obtained from 1 ml of overnight culture by using the Wizard Genomic DNA purification system (Promega Corporation, Madison, WI, USA), following the manufacturer’s instructions. Isolates were assayed for the presence of the gene tyrDC by PCR analysis with the primers DEC5 and DEC3 (Table 1), following the conditions described previously (Torriani et al., 2008). PCR product was visualized on a 2% agarose gel.

Randomly amplified polymorphic DNA (RAPD) analysis and identification of tyramine-positive cocci

In order to genetically typify the 35 tyramine-positive coccal strains, a preliminary RAPD-PCR analysis was performed with the primer 1254 (Table 1). Conversion, normalization and numerical analysis of the patterns were performed by GELCOMPAR 4.0 software (Applied Maths, Kortrijk, Belgium). A dendrogram was produced and major clusters with a cut-off point of about 60% in the UMPGA (Unweighted Pair Group Method with Arithmetic Averages) clustering analysis similarity level was taken as representing a single cluster. Species identification was carried out by phenylalanyl-tRNA synthase α-subunit (pheS) gene sequence analysis (Naser et al., 2005). The pheS partial gene amplification was obtained with the primers pheS-21-F and pheS-22-R (Table 1). PCR conditions were set according to Naser et al. (2005) with exception that annealing temperature was 50°C. The expected amplicon (455 bp) was purified with the Wizard SV gel and PCR clean-up system (Promega Corporation) and cloned with the cloning kit pGEMT-easy vector system (Promega Corporation). Recombinant plasmids were sequenced at the GATC Biotech Ltd (Koln, Germany). Data were analysed with the Basic Local Alignment Search Tool (BLAST) provided by National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Growth parameters of two Enterococcus mundtii strains and tyramine production

Two strains (C46 and C53), isolated from grass silage and identified as Enterococcus mundtii, were used for deeper investigations. The two considered enterococci were pre-cultivated for 24 h at 37°C in BHI broth added with 1000 mg l⁻¹ of tyrosine (Sigma-Aldrich, Gallarate, Italy). After 24 h of pre-cultivation, the microorganisms were inoculated, at a concentration of approximately 7 log CFU ml⁻¹, in BHI broth, added or not with 1 g l⁻¹ of tyrosine and incubated at 37°C for 72 h. The evaluation of the strain growth in BHI was performed by measuring the OD₆₀₀ with a UV-VIS spectrophotometer (Cary 60 UV-Vis; Agilent Technologies, Santa Clara, CA, USA) with plastic cuvettes (1.5 ml) at defined times (1, 2, 3, 4, 5, 6, 7, 8, 24, 48, 72 and 96 h). The OD₆₀₀ data were fitted with the Gompertz equation as modified by Zwietering et al. (1990).

\[
y = k + A e^{-e \left(\frac{t}{\lambda} - 1\right)}
\]

where \(y\) is the OD₆₀₀ at time \(t\), \(A\) represent the maximum OD₆₀₀ value reached, \(\lambda\) is the maximum OD₆₀₀ increase rate in exponential phase and \(\lambda\) is the lag time.

The maximum cell concentration reached was determined at the beginning of the stationary phase by plate counting enterococci onto BHI agar.

The BA were determined after 2, 3, 4, 5, 8, 24, 48, 72 and 96 h of incubation. The cultures were centrifuged at 10 000 rpm for 10 min at 10°C, and the supernatants were used for BAs determination by HPLC after derivatization with dansyl-chloride (Sigma-Aldrich, Gallarate, Italy). After 24 h of pre-cultivation, the microorganisms were inoculated, at a concentration of approximately 7 log CFU ml⁻¹, in BHI broth, added or not with 1 g l⁻¹ of tyrosine and incubated at 37°C for 72 h.
Tyramine production by Enterococcus mundtii

Statistical analysis

The growth model was fitted using the statistical package Statistica for Windows 6.1 (Statsoft Italia, Vigonza, Italy).

Conflict of interest

None declared.

References

Akopyanz, N., Bukanov, N.O., Westblom, T.U., Kresovich, S., and Berg, D.E. (1992) DNA diversity among clinical isolates of Helicobacter pylori detected by PCR-based RAPD fingerprinting. *Nucleic Acid Res* 20: 5137–5142.

Aymerich, T., Martin, B., Garriga, M., Vidal-Cardou, M.C., Bover-Cid, S., and Hugas, M. (2006) Safety properties and molecular strain typing of lactic acid bacteria from slightly fermented sausages. *J Appl Microbiol* 100: 40–49.

Bargossi, E., Gardini, F., Gatto, V., Montanari, C., Torriani, S., and Tabanelli, G. (2015a) The capability of tyramine production and correlation between phenotypic and genetic characteristics of *Enterococcus faecalis* and *Enterococcus faecalis* strains. *Front Microbiol* 6: 1371.

Bargossi, E., Tabanelli, G., Montanari, C., Lanciotti, R., Gatto, V., Gardini, F., and Torriani, S. (2015b) Tyrosine decarboxylase activity of enterococci grown in media with different nutritional potential: tyramine and 2-phenylethylamine accumulation and tyrDC gene expression. *Front Microbiol* 6: 259.

Beshkova, D., and Frengova, G. (2012) Bacteriocins from lactic acid bacteria: microorganisms of potential biotechnological importance for the dairy industry. *Engineer Life Sci* 12: 419–432.

Bigwood, T., Hudson, J.A., Cooney, J., McIntyre, L., Billington, C., Heinemann, J.A., and Wall, F. (2012) Inhibition of *Listeria monocytogenes* by *Enterococcus mundtii* isolated from soil. *Food Microbiol* 32: 354–360.

Bonetta, S., Bonetta, S., Carraro, E., Coisson, J.D., Travalgia, F., and Arlorio, M. (2008) Detection of biogenic amine producer bacteria in a typical Italian goat cheese. *J Food Prot* 71: 205–209.

Bover-Cid, S., and Holzapfel, W.H. (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int J Food Microbiol* 53: 33–41.

Collins, M.D., Farrow, J.A.E., and Jones, D. (1986) *Enterococcus mundtii* sp. nov. *Int J Syst Bacteriol* 36: 8–12.

Connif, N., Le Breton, Y., Dousset, X., Aufray, Y., Rincé, A., and Prévost, H. (2002) Identification of the *Enterococcus faecalis* tyrosine decarboxylase operon involved in tyramine production. *Appl Environ Microbiol* 68: 3537–3544.

Corsetti, A., Settanni, L., Chaves López, C., Felis, G.E., Mastrangelo, M., and Suzzi, G. (2007) A taxonomic survey of lactic acid bacteria isolated from wheat (*Triticum durum*) kernels and non-conventional flours. *Syst Appl Microbiol* 30: 561–571.

Coton, M., Coton, E., Lucas, P., and Lonvaud-Funel, A. (2004) Identification of the gene encoding a putative tyrosine decarboxylase of *Carnobacterium divergens* 508.
Development of molecular tools for the detection of tyramine-producing bacteria. *Food Microbiol* 21: 125–130.

De Kwaadsteniet, M., Todorov, S.D., Knoetze, H., and Dicks, L.M.T. (2005) Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15, with activity against Gram-positive and Gram-negative bacteria. *Int J Food Microbiol* 105: 433–444.

Espeche, M.C., Juárez Tomás, M.S., Wiese, B., Bru, E., and Nader-Macias, F.M.E. (2014) Physicochemical factors differentially affect the biomass and bacteriocin production by bovine *Enterococcus mundtii* CRL1656. *J Dairy Sci* 97: 779–797.

Feng, G., Guron, G.K.P., Churey, J.J., and Worobo, R.W. (2009) Characterization of Mundticin L, a class IIa anti-listeria bacteriocin from *Enterococcus mundtii* CUGF08. *Appl Environ Microbiol* 75: 5708–5713.

Fernández, M., and Zúñiga, M. (2006) Amino acid catabolic pathways of lactic acid bacteria. *Crit Rev Microbiol* 32: 155–183.

Fernández, M., Linares, D.M., and Alvarez, M.A. (2004) Sequencing of the tyrosine decarboxylase cluster of *Lactococcus lactis* IPLA 655 and the development of a PCR method for detecting tyrosine decarboxylating lactic acid bacteria. *J Food Prot* 67: 2521–2529.

Fernández, M., Linares, D.M., Rodríguez, A., and Alvarez, M.A. (2007) Factors affecting tyramine production in *Enterococcus durans* IPLA 655. *Appl Microbiol Biotechnol* 73: 1400–1406.

Fontana, C., Cocconcelli, P.S., Vignolo, G., and Saavedra, L. (2015) Occurrence of antilisterial structural bacteriocins in meat borne lactic acid bacteria. *Food Control* 47: 53–59.

Foulquié Moreno, M.R., Sarantinopoulos, P., Tsakalidou, E., and De Vuyst, L. (2006) The role and application of enterococci in food and health. *Int J Food Microbiol* 106: 1–24.

Franz, C.M.A.P., Holzapfel, W.H., and Stiles, M.E. (1999) Enterococci at the crossroads of food safety? *Int J Food Microbiol* 47: 1–24.

Franz, C.M.A.P., Stiles, M.E., Schliefer, H.K., and Holzapfel, W. (2003) Enterococci in food-a conundrum for food safety. *Int J Food Microbiol* 88: 105–122.

Franz, C.M.A.P., Huch, M., Abriouel, H., Holzapfel, W., and Gálvez, A. (2011) Enterococci as probiotics and their implications in food safety. *Int J Food Microbiol* 151: 125–140.

Furtula, V., Jackson, C.R., Farrell, E.G., Barrett, J.B., Hiot, L.M., and Chambers, P.A. (2013) antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *Int J Environ Res Public Health* 10: 1020–1036.

Gardini, F., Bover-Cid, S., Tofalo, R., Belletti, N., Gatto, V., Suzzi, G., and Torriani, S. (2008) Modeling the amino- genetic potential of *Enterococcus faecalis* EF37 in dry fermented sausages through chemical and molecular approaches. *Appl Environ Microbiol* 74: 2740–2750.

Giraffa, G. (2002) Enterococci from foods. *FEMS Microbiol Rev* 26: 163–171.

Giraffa, G. (2003) Functionality of enterococci in dairy products. *Int J Food Microbiol* 88: 215–222.

Graves, A.K., and Weaver, R.W. (2010) Characterization of enterococci populations collected from a subsurface flow constructed wetland. *J Appl Microbiol* 108: 1226–1234.

Higashide, T., Takahashi, M., Kobayashi, A., Ohkubo, S., Sakurai, M., Shirao, Y., et al. (2005) Endophthalmitis caused by *Enterococcus mundtii*. *J Clin Microbiol* 43: 1475–1476.

Hollenbeck, B.L., and Rice, L.B. (2012) Intrinsically and acquired resistance mechanisms in enterococci. *Virology* 3: 421–433.

Kalhotka, L., Manga, I., Prichystalová, J., Hůlová, M., Vyleťelová, M., and Sustová, K. (2012) Decarboxylase activity test of the genus *Enterococcus* isolated from goat milk and cheese. *Acta Vet Brno* 81: 145–151.

Klein, G. (2003) Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastro-intestinal tract. *Int J Food Microbiol* 88: 123–131.

Komporda, T., Burdycechová, R., Dohnal, V., Ciková, O., and Sládek, P. (2008) Some factors influencing biogenic amines and polyamines content in Dutch-type semi-hard cheese. *Eur Food Res Technol* 227: 29–36.

Ladero, V., Fernández, M., Calles-Enríquez, M., Sánchez-Llana, E., Cañedo, E., Martin, M.C., and Alvarez, M.A. (2012) Is the production of the biogenic amines tyramine and putrescine a species-level trait in enterococci? *Food Microbiol* 30: 132–138.

Leclerc, H., Devries, L.A., and Mose, D.A.A. (1996) Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water. *J Appl Bacteriol* 81: 459–466.

Leuschner, R.G.K., Kunhara, R., and Hammes, W.P. (1999) Formation of biogenic amines by proteolytic enterococci during ripening. *J Sci Food Agric* 79: 1141–1144.

Linares, D.M., Fernández, M., Martin, M.C., and Alvarez, M.A. (2009) Tyramine biosynthesis in *Enterococcus durans* is transcriptionally regulated by the extracellular pH and tyrosine concentration. *Microb Biotechnol* 2: 625–633.

Linares, D.M., Martin, M.C., Ladero, V., Alvarez, M.A., and Fernández, M. (2011) Biogenic amines in dairy products. *Crit Rev Food Sci Nutr* 51: 691–703.

Linares, D.M., del Rio, B., Bedrullo, B., Ladero, V., Cruz, M., de Jong, A., et al. (2015) AguR, a transmembrane transcription activator of the putrescine biosynthesis operon in *Lactococcus lactis*, acts in response to the agmatine concentration. *Appl Environ Microbiol* 81: 6145–6157.

Liu, F., Du, L., Xu, W., Wang, D., Zhang, M., Zhu, Y., and Xu, W. (2013) Production of tyramine by *Enterococcus faecalis* strains in water-boiled salted duck. *J Food Prot* 76: 854–859.

Lucas, P., Landete, J., Coton, M., Coton, E., and Lonvaud-Funel, A. (2003) The tyrosine decarboxylase operon of *Lactobacillus brevis* IOB9809: characterization and conservation in tyramine-producing bacteria. *FEMS Microbiol Lett* 229: 65–71.

Lund, P., Tramonti, A., and De Blase, D. (2014) Coping with low pH: molecular strategies in neutralophilic bacteria. *FEMS Microbiol Rev* 38: 1–35.

Lynch, M., and Conery, J.S. (2000) The evolutionary fate and consequences of duplicate genes. *Science* 290: 1151–1155.

Marcobal, A., De Las Rivas, B., and Muñoz, R. (2006) First genetic characterization of a bacterial -phenylethylamine
biosynthetic enzyme in Enterococcus faecium RM58. FEMS Microbiol Lett 258: 144–149.
Marcobal, A., De Las Rivas, B., Landete, J.M., Tabera, L., and Muñoz, R. (2012) Tyramine and phenylethylamine biosynthesis by food bacteria. Crit Rev Food Sci Nutrit 52: 448–467.
McCabe-Sellers, B., Staggs, C.G., and Bogle, M.L. (2006) Tyramine in foods and monoamine oxidase inhibitor drugs: a crossroad where medicine, nutrition, pharmacy, and food industry converge. J Food Comp Anal 19: S58–S65.
Molenaar, D., Bosscher, J.S., ten Brink, B., Driessen, A.J., and Konings, W.N. (1993) Generation of a proton motive force by histidine decarboxylase and electrogenic histidine/histamine antiport in Lactobacillus buchneri. J Bacteriol 175: 2864–2870.
Moore, D.F., Guzman, J.A., and McGee, C. (2008) Species distribution and antimicrobial resistance of enterococci isolated from surface and ocean water. J Appl Microbiol 105: 1017–1025.
Muller, T., Ulrich, A., Ott, E.M., and Muller, M. (2001) Identification of plant-associated enterococci. J Appl Microbiol 91: 268–278.
Naser, S.M., Thompson, F.L., Hoste, B., Gevers, D., Moore, D.F., Guzman, J.A., and McGee, C. (2008) Species distribution and antimicrobial resistance of enterococci isolated from surface and ocean water. J Appl Microbiol 105: 1017–1025.
Muller, T., Ulrich, A., Ott, E.M., and Muller, M. (2001) Identification of plant-associated enterococci. J Appl Microbiol 91: 268–278.
Naser, S.M., Thompson, F.L., Hoste, B., Gevers, D., Dawyndt, P., Vancanneyt, M., and Swings, J. (2005) Application of multilocus sequence analysis (MLSA) for rapid identification of Enterococcus species based on rpoA and phoE genes. Microbiol 151: 2141–2150.
Ni, K., Wang, Y., Li, D., Cai, Y., and Pang, H. (2015) Characterization, identification and application of lactic acid bacteria isolated from forage paddie rice silage. PLoS ONE 10: e0121967.
Pereira, C.I., Matos, D., San Roma, M.V., and Barreto Crespo, M.T. (2009) Dual role for the tyrosine decarboxylation pathway in Enterococcus faecium E17: response to an acid challenge. Appl Environ Microbiol 75: 345–352.
Perez, M., Calles-Enriquez, M., Nes, I., Martin, M.C., Fernandez, M., Ladero, V., and Alvarez, M.A. (2015) Tyramine biosynthesis is transcriptionally induced at low pH and improves the fitness of Enterococcus faecalis in acidic environments. Appl Microbiol Biotechnol 99: 3547–3558.
Pérez-Martín, F., Sesena, S., Miguel Izquierdo, P., and Palop, M.L. (2014) Are Enterococcus populations present during malolactic fermentation of red wine safe? Food Microbiol 42: 95–101.
Repizo, G.D., Espariz, M., Blancato, V.S., Suárez, C.A., Esteban, L., and Magni, C. (2014) Genomic comparative analysis of the environmental Enterococcus mundtii against enterococcal representative species. BMC Genom 15: 489.
Romano, A., Ladero, V., Alvarez, M.A., and Lucas, P.M. (2014) Putrescine production via the ornithine decarboxylation pathway improves the acid stress survival of Lactobacillus brevis and is part of a horizontally transferred acid resistance locus. Int J Food Microbiol 175: 14–19.
Rossi, F., Rizzotti, L., Felis, G.E., and Torriani, S. (2014) Horizontal gene transfer among microorganisms in food: current knowledge and future perspective. Food Microbiol 42: 232–243.
Schöbitz, R., González, C., Villarreal, K., Horzella, M., Nahuelquín, Y., and Fuentes, R. (2014) A biocontroller to eliminate Listeria monocytogenes from the food processing environment. Food Control 36: 217–223.
Shalaby, A.R. (1994) Significance of biogenic amines to food safety and human health. Food Res Int 29: 675–690.
Shiwa, Y., Yanase, H., Hirose, Y., Satomi, S., Araya-Kojima, T., Watanabe, S., et al. (2014) Complete genome sequence of Enterococcus mundtii QU 25, an efficient L-(1)-lactic acid-producing bacterium. DNA Res 21: 369–377.
Sivers, F., Wilm, A., Dineen, D.G., Gibson, T.J., Karplus, K., Li, W., et al. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7: 539. doi:10.1038/msb.2011.75.
Solovjev, V., and Salamov, A. (2011) Automatic annotation of microbial genomes and metagenomic sequences. In Metagenomics and Its Applications in Agriculture, Biomedicine and Environmental Studies. Li, R.W. (ed). New York: Nova Science Publishers, pp. 61–78.
Suzzi, G., and Gardini, F. (2003) Biogenic amines in dry fermented sausages: a review. Int J Food Microbiol 88: 41–54.
Tabanelli, G., Coloretti, F., Chiavari, C., Grazia, L., Lanciotti, R., and Gardini, F. (2012) Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. Food Control 26: 416–426.
Todorov, S.D., Wachsmann, M.B., Knoetze, H., Meincken, M., and Dicks, L.M.T. (2005) An antibacterial and antiviral peptide produced by Enterococcus mundtii ST4V isolated from soya beans. Int J Antimicrob Agents 25: 508–513.
Torriani, S., Gatto, V., Sembeni, S., Tofalo, R., Suzzi, G., Belletti, N., et al. (2008) Rapid detection and quantification of tyrosine decarboxylase gene (tdc) and its expression in gram-positive bacteria associated with fermented foods using PCR-based methods. J Food Prot 71: 93–101.
Trivedi, K., Borkovcová, I., and Karpíšková, R. (2009) Tyramine production by enterococci from various foodstuffs: a threat to the consumers. Czech J Food Sci 27: S357–S360.
Vancanneyt, M., Lombardi, A., Andrighetto, C., Knijff, E., Torriani, S., Bjarkroth, K.J., et al. (2002) Intraspecies genomic groups in Enterococcus faecium and their correlation with origin and pathogenicity. Appl Environ Microb 68: 1381–1391.
Vera Pingitore, E., Todorov, S.D., Sesma, F., and Franco, B.D. (2012) Application of bacteriocinogenic Enterococcus mundtii CRL35 and Enterococcus faecium ST88Ch in the control of Listeria monocytogenes in fresh Minas cheese. Food Microbiol 32: 38–47.
Wright, A., and Axelsson, L. (2012) Lactic acid bacteria: an introduction. In Lactic Acid Bacteria: Microbiological and Functional Aspects, 4th ed. Lahtinen, S., Ouwehand, A.C., Salminen, S., and von Wright, A. (eds). Boca Raton, FL, USA: CRC Press, Tayor & Francis Group, pp. 1–16.
Zwietering, M.H., Jongenburger, I., Rombouts, F.M., and van’t Riet, K. (1990). Modelling of the bacterial growth curve. Appl Environ Microb 56: 1875–1881.