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Occurrence of the structural enterocin A, P, B, L50B genes in enterococci of different origin

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Abstract

Enterococci are well-known producers of antimicrobial peptides—bacteriocins (enterocins) and the number of characterized enterocins has been significantly increased. Recently, enterocins are of great interest for their potential as biopreservatives in food or feed while research on enterocins as alternative antimicrobials in humans and animals is only at the beginning. The present study provides a survey about the occurrence of enterocin structural genes A, P, B, L50B in a target of 427 strains of *Enterococcus faecium* (368) and *Enterococcus faecalis* (59) species from different sources (animal isolates, food and feed) performed by PCR method. Based on our results, 234 strains possessed one or more enterocin structural gene(s). The genes of enterocin P and enterocin A were the most frequently detected structural genes among the PCR positive strains (170 and 155 strains, respectively). Different frequency of the enterocin genes occurrence was detected in strains according to their origin; the strains from horses and silage showed the highest frequency of enterocin genes presence. All possible combinations of the tested genes occurred at least twice except the combination of the gene of enterocin B and L50B which possessed neither strain. The gene of enterocin A was exclusively detected among *E. faecium* strains, while the gene of enterocin P, B, L50B were detected in strains of both species *E. faecium* and *E. faecalis*. In conclusion, a high-frequency and variability of enterocin structural genes exists among enterococci of different origin what offers a big possibility to find effective bacteriocin-producing strains for their application in veterinary medicine.

Keywords: Bacteriocin; Bacteriocin structural genes; *Enterococcus* sp.; Enterocin; PCR

1. Introduction

The ability of enterococci to produce ribosomally synthesized, extracellularly released antimicrobial peptides—bacteriocins is well known (Nes et al., 1996). According to the classification scheme of Franz et al. (2007), enterocins belong to class I (lantibiotic enterocins), class II (small, nonlantibiotic peptides), class III (cyclic enterocins) and class IV (large proteins). Class II is subdivided in three subclasses: II. 1, enterocins of the pediocin family, II. 2, enterocins synthesized without a leader peptide and II. 3, other

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linear, non-pediocin type enterocins. Up to now, the best characterized enterocins are enterocin A (Aymerich et al., 1996), P (Cintas et al., 1997), B (Casaus et al., 1997), L50A and L50B (Cintas et al., 1998), I (Floriano et al., 1998) and Q (Cintas et al., 2000). Moreover, the information on enterocin M was already published (Mareková et al., 2007). Enterocins are most frequently produced by *Enterococcus faecium* strains, however, other species of enterococci have also been found to produce bacteriocins, e.g. *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus mundtii*, *Enterococcus durans*, *Enterococcus avium*, *Enterococcus gallinarum*, *Enterococcus casseliflavus* and *Enterococcus columbae* (Jennes et al., 2000; Sabia et al., 2004; Sánchez et al., 2007).

Although, the efficiency of enterocins against food spoilage and pathogenic bacteria (*Listeria* sp., *Staphylococcus* sp., *Bacillus* sp., *Clostridium* sp., *Escherichia coli*) in various food systems is well demonstrated (Aymerich et al., 2000; Lauková and Czikková, 2001), only little information is available on the role of bacteriocins in the animal ecosystem. It is assumed that bacteriocin production is a bacterial defense mechanism, which gives the producer strain a competitive advantage towards non-producer and bacteriocin-sensitive strains in the same niche (Chen and Hoover, 2003). Several studies indicated applicability of enterocins or enterocin-producing strains to control/reduce pathogenic bacteria in the gastrointestinal tract of animals (*Salmonella* sp., *Campylobacter* sp., *E. coli*; Lauková et al., 2003; Cole et al., 2006). In addition, bacteriocins of other bacterial genera showed potential to prevent bovine mastitis (Meaney et al., 2001) and gingivitis (Howell et al., 1993), but also to modulate the immune system (De Pablo et al., 1999) or the rumen fermentation (Callaway et al., 1997). Furthermore, the antitumorous activity was reported (Hill and Farkas-Himsley, 1991).

This study provides data on the occurrence of enterocin structural genes A, P, B, L50B (belong to firstly characterized enterocins in detail) as well as on the presence of various genes combinations in a target of 427 enterococci isolated from different sources. It compares the distribution of these genes in the three main environments—animals, food and feed.

2. Materials and methods

2.1. Bacterial strains

A collection of 427 *Enterococcus* strains were isolated using M-*Enterococcus* agar (Becton and Dickinson, Cockeysville, USA). Species identification of the strains (mostly isolates of Laboratory of Animal Microbiology, Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovakia) was performed by PCR method as well as by tDNA-PCR (Welsh and McClelland, 1991; Baele et al., 2001) and strains were allotted to the species *E. faecium* (368) and *E. faecalis* (59) (Lauková and Mareková, 2001; Strompfová et al., 2004; Marciňáková et al., 2005; Simonová, 2006). Strains were collected from the following animals: chicken (wall and content of crop, ileum and caecum; $n = 15$), dog (faeces, $n = 10$), horse (faeces, $n = 12$), pig (rectal swabs, $n = 12$), rabbit (faeces, $n = 15$), rodent (intestinal content of *Clethrionomys glareolus*, *Microtus oeconomus* P., $n = 9$, provided by Dr. I. Swiecicka, Poland), ruminants domestic (rumen content, faeces of calf $n = 8$, lamb $n = 6$, goat $n = 10$) and from wild ruminants (rumen content of *Rupicapra rupicapra tatrica*, $n = 8$, *Cervus elaphus carpaticus*, $n = 8$). The strains of food origin were isolated from fermented meat products (French, Italian and Slovak traditional fermented sausages), French and Italian strains were provided by the partners of EU project QLK1-CT-2002-02240. The source of strains collected from feed was grass silage ($n = 10$) and commercial granulated feed for dogs ($n = 12$).

2.2. PCR detection of enterocin structural genes

DNA was isolated by rapid alkaline lysis method as described by Baele et al. (2001). Following sequences of primers were used: 5′-GAGATTTATCTCCATAATCT-3′ and 5′-GTACCACTCATAGTGGAA-3′ for enterocin A (Aymerich et al., 1996), 5′-ATGAGAAAAAAATTATTTAGTTT-3′ and 5′-TTAATGTCCCATACCTGCCAAACC-3′ for enterocin P (Cintas et al., 1997), 5′-GAAAATGATCACAGAATGCCTA-3′ and 5′-GTTGCATTTAGAGTATACATTTG-3′ for enterocin B (Casaus et al., 1997), 5′-ATGGGAGCAATCGCAAAATTA-3′ and 5′-TAGCCATTTTTCAATTTGATC-3′ for enterocin L50B