Identification of *Enterococcus* sp. in GIT of Broiler Chickens after Application of Biological Preparations

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Abstract
The aim of the present study was a rapid detection and identification of *Enterococcus* sp. in various segments of chicken gastrointestinal tract by polymerase chain reaction (PCR) analysis. As a biological material were used broiler chickens Hybro. They were fattening by the combined probiotic preparation for elimination of pathogens and better utilization of feed. In our study, the identification of *Enterococcus species* was based on the superoxid dismutase gene (*sodA*). *Enterococcus faecium, Enterococcus faecalis* were determined in all samples (100% occurence). Occurence of *Enterococcus gallinarum* was 87.5% and *Enterococcus cecorum* was 0%.

Key words: *Enterococcus*, chicken, detection, polymerase chain reaction

1. Introduction

Enteric diseases are an important concern to the poultry industry because of decreasing productivity, increased mortality and the associated contamination of poultry products for human consumption (human food safety). Prebiotics and probiotics are two of several approaches that have potential to reduce enteric disease in poultry and subsequent contamination of poultry products.
In broiler nutrition, probiotic species belonging to *Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus* and *Saccharomyces* have a beneficial effect on broiler performance [1,18], modulation of intestinal microflora and pathogen inhibition [8,9,14,15], immunomodulation [11,12,19], intestinal histological changes [2,10,16], improving sensory characteristics of broiler meat and promoting microbiological meat quality of broilers [6,7]. Therefore, considerations concerning the taxonomy, ecology and antibiotic resistance of enterococci are important for the understanding of the widespread occurrence of these organisms and also for the selection and characterisation of commercially used enterococcal strains. Molecular approaches identifying changes in specific bacterial populations or general changes in microbial community structure should enhance our understanding of intestinal microbial ecology, including the influence of probiotics and prebiotics. In our study, the molecular identification of *Enterococcus* species is based on the superoxid dismutase gene (*sodA*).

2. Materials and methods

As biological material were used one day old chickens (both sexes) of meat type Hybro. They were treated for 35 days by the probiotic preparation based on *Lactobacillus delbrueckii ssp. bulgaricus* LAT187, *Lactobacillus helvetius* LAT179, *Lactobacillus acidophilus* LAT180, *Lactobacillus delbrueckii ssp.lactis* LAT182, *Streptococcus termophilus* LAT205, *Enterococcus faecium* E-253.
Sample collection. The birds were killed by cervical dislocation, and GI tracts were removed,

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placed in sterile plastic bags, and immediately immersed in ice for transport to the laboratory.
Four types of samples (crop, gizzard, duodeum, ceacum) were prepared for microbial cultivation.
Microbiology. Chyme contents were streaked directly onto Slanetz Bartley Agar (Biomark, Pune, IND) and incubated 48 hours at 37°C. After incubation, colonies formed on the respective media were carefully observed, counted and picked up for Gram staining. At least ten *Enterococcus* sp. colonies were selected from each sample and incubated (overnight at 37°C) in Peptone water. 2 ml of solution was used for isolation of bacterial DNA.
DNA isolation. DNA was extracted with GenElute ^{TM} Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, USA).
PCR. Genus primers designed by Deasy et al. [3] were used in each reaction to confirm the genus enterococci. Four PCR master mixes (Table 1.) consisting of different primer set were prepared.

Table 1. Bacterial strains used for PCR

master mix	Enterococcus sp.	Product size (bp)
mix 1	*E. faecium*	550
mix 2	*E. faecalis*	941
mix 3	*E. gallinarum*	173
mix 4	*E. cecorum*	317

The base master mix consisted of 5 µl 10x Restorase reaction buffer, 0,05 U.µl^{-1} Restorase DNA Polymerase (Sigma-Aldrich, St. Louis, USA), 1 µl dNTP Mix (10mM), H_2O (redistilled) in addition to final volume and 1 µl (2 µM) each genus primer. PCRs were performed in a final volume of 50 µl consisting of 1 – 2 ng. µl^{-1} DNA template. Following an initial denaturation at 95°C for 4 min, products were amplified by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min, and elongation at 72°C for 1 min. Amplification was followed by a final extension at 72°C for 7 min. PCR products were electrophoresed on a 2% Tris-acetate-EDTA agarose gel and stained in GoldView^{TM}.

3. Results and discussion

The major niche of entrococci is the gastrointestinal tract (GIT) of human and animal where they make up a significant portion of the normal microflora. In our study we were focused to isolate the major species of *Enterococcus* genus in various GIT segments (crop, gizzard, duodenal loop and ceaces) of broilers. The four enterococcal species: *E. faecium, E. faecalis, E. gallinarum* and *E. cecorum* were isolated from broilers on the end of feeding. The PCR method using *sodA* gene which catalyzes the dismutation of superoxide shoved that in some cases distinct bands were not evident for *E. cecorum* and *E. gallinarum*. On the other hand, a distinct DNA band for *E. faecium and E. faecalis* was observed. These organisms covered 100% of the total isolated enterococci. *E. gallinarum* was the most frequently identified *Enterococcus* spp. (87.5%) especially in ceaces (Figure 1). We have compared our result with others authors [4,13,17]. They are very similar in isolated species.

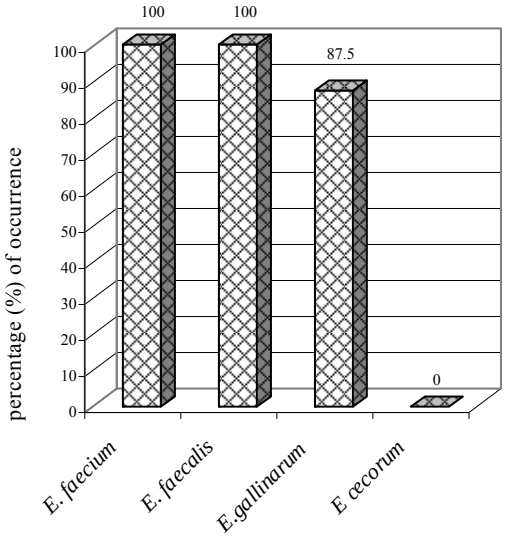

Figure 1. Occurence of *Enterococcus sp.* in GIT of chickens

Occurence of *E. cecorum* was 0 %. This fact can be explain, that *E. cecorum* is predominant in the intestine of chickens over 12 weeks old [5].
The microbial community of the gastrointestinal tract ultimately reflects the co-evolution of microorganisms with their animal host and the diet adopted by the host. Changes in composition of the animal microflora can have determinal effect on health, growth and maturation of the animal host [13].
Stabilization of intestinal microflora can be supported by biological preparations such as probiotic and prebiotic. Probiotics strains are often lactic acid producing bacteria (LAB), and

Enterococcus species are considered as a part of them. They are very resistant to surrounding condition and the have high viability. Species from genus of *Enterococcus* are appropriate for use like a probiotic preparations, especially *E faecium*. The high rate of *E. faecium* isolates can confirm this presupposition.

4. Conclusions

The molecular techniques for the more accurate identification of intestinal microflora help define the function of commensally bacteria in the GIT. A previous report identified the manganese-dependent superoxide dismutase gene *sodA* as an ideal gene for species identification of enterococci. Simple PCR provides an accurate and quick method for identification of enterococci, without the need for extensive phenotypic tests.

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