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Influence of intensive and extensive breeding on lactic acid bacteria isolated from *Gallus gallus domesticus* ceca

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Abstract

In the present study, lactic acid bacteria (LAB) from the cecum of chickens bred either under intensive (commercial broilers) or extensive (free-range) conditions were isolated, identified and some of their probiotic characteristics determined. The LAB identified by 16S–23S rRNA PCR-ARDRA were mainly of *Lactobacillus* species and to a lesser extent of *Enterococcus* spp. for all animals. Free-range chickens showed a higher presence of *Lactobacillus acidophilus* while *Lactobacillus reuteri* and *Lactobacillus johnsonii* were more frequently recovered from commercial broilers. *Lactobacillus crispatus* was found only in commercial broilers, *Lactobacillus vaginalis* and *Lactobacillus agilis* only in free-range chickens and *Lactobacillus salivarius* in both types. *Enterococcus* isolates from ceca of commercial broilers showed a higher resistance to antimicrobial drugs. *Lactobacillus* isolates from free-range chickens presented a higher frequency of *in vitro* antagonistic activity against selected pathogens than from commercial broilers. All LAB isolates had predominantly non-hydrophobic surfaces, but with variations depending on age of the chickens and breeding conditions. Animal breeding caused variation on composition, antimicrobial susceptibility, antagonistic activity and surface hydrophobicity of LAB from chicken cecum. LAB isolates from ceca of free-range chickens have potential as probiotic agents, which may be used in the future as replacing the use of antimicrobials as growth promoters.

Keywords: Lactic acid bacteria; Microbiota; Ceca; Free-range chickens; Broiler chickens; Probiotics

1. Introduction

The gastrointestinal microbiota plays an important role in nutrition, detoxification of certain compounds, growth performance, and protection against infection

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(McCracken and Lorenz, 2001). Lactic acid bacteria (LAB) colonize in high population levels (higher than 10^7 viable cells per gram of contents) the gastrointestinal tract of broiler chickens, but LAB species differ depending on the anatomical site (Zhu and Joerger, 2003). The lactobacilli are predominant populations in association with other bacterial genera in the cecum (Zhu et al., 2002), but alone in the crop (Guan et al., 2003) and the ileum (Knarreborg et al., 2002).

In Brazil, commercial broiler chickens are reared in large-scale farms and fed with growth promoters. Free-range chickens (''caipira'') are not raised according to modern breeding technologies and eat only what they find on the ground and corn grains.

The breeding environment and feeding are important factors in determining the intestinal microbial communities (Knarreborg et al., 2002). Growth promoters in the feed alter the intestinal microbiota and induce a dissemination of antimicrobial resistance (WHO/FAO/OIE, 2003). Hence, there is an increasing interest in developing alternative methods of controlling the gastrointestinal microbial ecosystem, enhancing the growth of indígenous beneficial bacteria (i.e., prebiotics) or introducing viable bacteria (i.e., probiotics) that benefit the host (Nashashon et al., 1996). Probiotics are preferentially isolated from the gastrointestinal microbiota of the animal species of interest and frequently selected among LAB. Sensitivity to antimicrobials (to avoid resistance transfer), production of inhibitory substances (to antagonize pathogenic microorganisms) and hydrophobic cell wall (to facilitate adhesion to intestinal epithelium) are other desirable properties for probiotic use.

Because the gastrointestinal microbiota in Brazilian ''caipira'' chickens is not known, the objectives of the present work were to determine the influence of intensive or extensive breeding conditions on LAB composition in ceca of chickens (*Gallus gallus domesticus*) and to evaluate probiotic properties of these bacteria.

2. Materials and methods

2.1. Animals

Ten Cobb commercial broilers (*Gallus gallus domesticus*) intensively raised in a large-scale chicken farm were used. Five of them were 4 days old and other five 45 days old. The feed varied according to age (pre-initial 1–7 days; initial 8–21 days; growth 22–40 days and finishing 41–45 days old) and contained corn, soy meal, degommed soy meal, bone and meat meal, salt and vitamin premixes. The first three age stages also received coccidiostatics. The manufacturer did not identify the antimicrobials present in the feed.

Ten free-range chickens (*Gallus gallus domesticus*), five 14-day-old and other five 180-day-old, bred under extensive conditions were also used. They did not have a specific breed, being the result of several crossings among indigenous farm chickens. Feeding was based on corn grain, grass, vegetable wastes, insects, ticks and earthworms. No antimicrobial drug was used.

2.2. Isolation and physiological characterization of LAB

The animals were transported to the laboratory and immediately sacrificed by cervical dislocation. Cecum was removed under aseptic conditions inside a laminar flow hood (VECO, Campinas, Brazil). Luminal content and mucous scraping of each fowl were recovered, weighed and submitted to serial decimal dilution in buffered saline (5.61 g NaCl; 1 g KH_2PO_4; 2 g Na_2HPO_4 and 0.11 g KCl in 1000 ml distillated water) up to 10^{-5}. Materials were introduced immediately into an anaerobic chamber (Forma Scientific Company, Marietta, USA, containing an atmosphere of N_2 85%, H_2 10% and CO_2 5%) and 0.1 ml of each dilution was spread onto plates containing Man, Rogosa and Sharp (MRS) agar (Merck, Darmstadt, Germany). The plates were incubated in the anaerobic chamber for 48 h at 37 °C. Each colony presenting distinct morphology was isolated, stained by Gram and tested for catalase. Respiratory tests under aerobic, microaerophilic and anaerobic conditions were also performed using MRS agar (Difco, Sparks, USA) incubated during 48 h at 37 °C. Finally, the isolated microorganisms were inoculated into 5 ml MRS broth (Difco) and anaerobically incubated for 48 h at 37 °C. After growth, 500 μl of the broth were transferred to Eppendorf tubes containing 50 μl of sterile glycerol before freezing at −18 °C. The remaining broth was used for molecular identification of the isolated bacteria.