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Canine Lactic Acid Bacteria Identification and Selection as Potential Probiotic to use as Oral Vaccine Vehicle

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Lactobacilos are Gram-positive bacteria, catalase negative, capable of fermenting glucose and other sugars with its final major product been lactic acid. They must have several characteristics to make them potentially good candidates as antigenic vehicles for oral vaccines and as good probiotics. Seventy nine (79) bacteria were isolated from the feces of Chinese Crested and Yorkshire Terrier puppies, around 20 days old, and were submitted to molecular identification at the genus level. Of these 79 bacteria previously isolated, thirty seven (37) isolates were first typed at species level as lactobacilos by PCR amplification of 16S-23S rDNA intergenic spacers using universal primers that anneal within 16S and 23S genes, followed by restriction digestion analyses of PCR amplicons (PCR-ARDRA) and were submitted to probiotic characterization. Species identification by 16S-23S rRNA ARDRA showed that ten different species were recovered in the following order: *L. reuteri* (52%), *L. fructivorans* (12%), *L. animalis* (9%), *L. murinus* (6%), *L. sanfrascis-censis* (6%), *L. paraplantarum* (3%), *L. acidophilus* (3%), *L. salivarius* (3%), *L. sakei* (3%), e *L. paralimentarius* (3%). Seven (7) isolates were not identified trough 16S-23S rDNA ARDRA. These bacteria were submitted to sequencing reaction of 16S rDNA region. The sequencing reaction was performed by dideoxi method (SANGER et al., 1977) by an auto-matic capilary sequencer MegaBase 1000 (APBiotech). The sequencing reaction reagent used was Dyenamic ET Dye terminator cycle sequencing kit (Amersham Biosciences) and specific sense and anti sense primers. The other seven (7) isolates were recovered in the following order: 2 (*L. reuteri*), 17 (*Enterococcus hirae*), 18 (*L. animalis*), 19 (*Enterococcus faecium*) 20 (*Enterococcus faecalis*), 21 (*L. reuteri*) e 22 (*Enterococcus hirae*). The isolates 17, 19, 20 e 22 were classified as another genus, called enterococci, they should had been excluded by 16S-23S rDNA amplification reaction and by gram test too. The isolate number 15 had been identified by 16S-23S rRNA PCR-ARDRA as *L. plantarum* A, but it was identified latter as *L. paraplantarum* by multiplex PCR of *rec*A gene (Torriani *et al.* 2001). The most prevalent specie found in this canine fecal samples was *L. reuteri*, corresponding to more than 50% of the isolates. From the species found in this study, *L. salivarius*, *L. reuteri* e *L. murinus* had already been identified in canine feces by others authors in literature (Fujisawa e Mitsuoka, 1996; Greetham *et al.* 2002).